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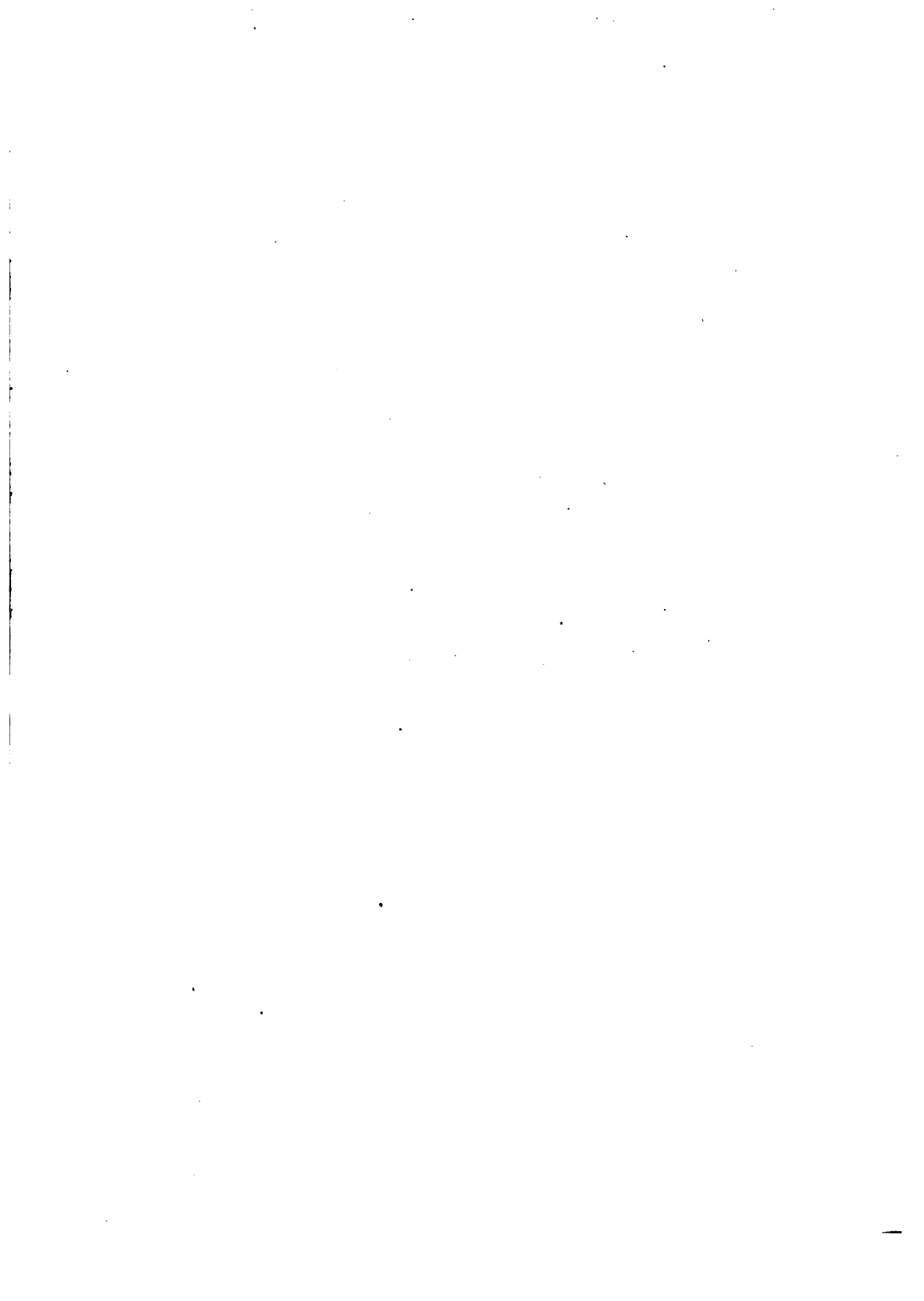
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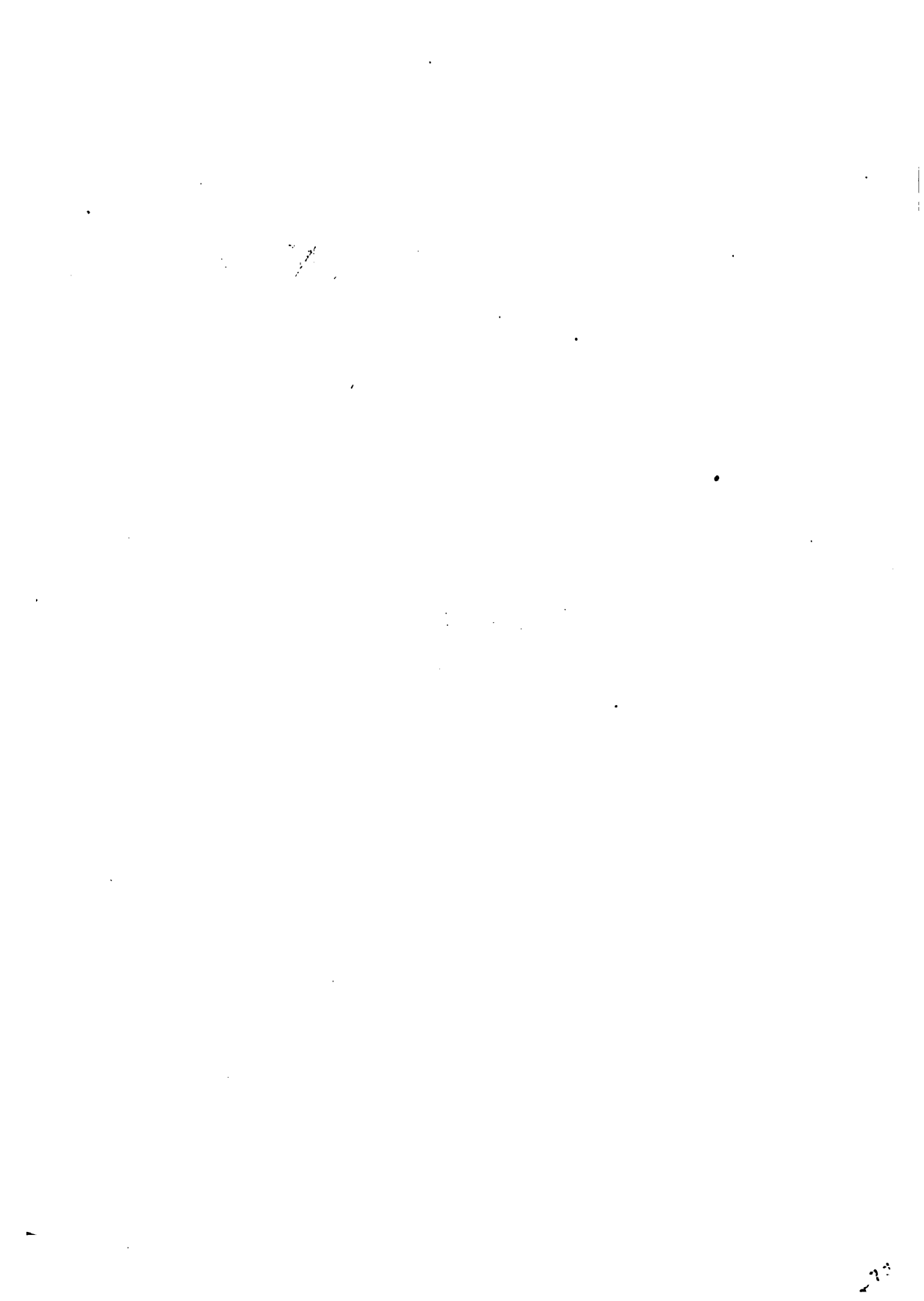




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## GASTRIC RESPONSE TO FOODS<sup>1</sup>

X. THE PSYCHIC SECRETION OF GASTRIC JUICE IN NORMAL MEN  
RAYMOND J. MILLER, OLAF BERGEIM, MARTIN E. REHFUSS AND  
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As early as 1852 it was shown by Bidder and Schmidt (1) that the mere sight of food called forth the secretion of gastric juice in the dog. It remained, however, for Pavlov (2), some forty years later, to establish more definitely the character of the so-called "appetite" or "psychic" secretion of the gastric juice. He pointed out that such a secretion was the normal initiator of gastric digestion in dogs, and might be induced by the sight, smell, taste, mastication or thought of food, or even through the stimulation of appetite by the presence of solid matter in the stomach. This was further emphasized by the fact that very appetizing foods, such as meats, induced a greater secretion in "sham feeding" experiments than milk or bread, which were not so greatly relished by the animals.

The results of experiments on man have been by no means so conclusive. Thus Carlson (3) was able to point out probable sources of error in most of the earlier investigations. This author concluded from the work of Homborg (4) and his own experiments that in spite of apparent evidence in the literature to the contrary, what Pavlov found true for animals was also true for man, namely, that gastric secretion was not induced by the chewing of indifferent substances nor by the taste or smell of chemical substances not arousing the appetite sensation.

<sup>1</sup> The expenses of this investigation were defrayed by funds furnished by Mrs. M. H. Henderson, The Curtis Publishing Company and Dr. L. M. Halsey.

Accepting the view that the initiation of gastric secretion is dependent upon arousing or augmenting the appetite sensation, it becomes of immediate interest to determine whether the thought, sight, smell or taste of food exerts the greatest influence in this direction as well as to estimate the absolute importance of the appetite secretion as a factor in gastric digestion.

Carlson found that no secretion could be induced in his gastric fistula subject by the thought of food, and that the secretion produced by seeing or smelling food was relatively slight and inconstant, the significant appetite secretion being that induced by the tasting and chewing of good food. Luckhardt (5), on the contrary, employing a Rehfuß stomach tube and using as a subject a completely normal man, found under good experimental conditions that the combined sight and smell of food markedly increased the flow of gastric juice.

Pavlov believed the appetite secretion to be of very great importance in initiating gastric digestion. This is discounted by Carlson, who found in his human subject, as well as in cats and dogs, that the continuous secretion of the stomach served a similar purpose, and that elimination of the appetite secretion did not cause indigestion.

That the unpalatable condition of a food need not necessarily influence its ultimate digestion and utilization in the alimentary tracts of normal men was indicated by work carried out in the laboratory of Atwater, who found that nauseating meat, the so-called "embalmed beef" fed to soldiers in the Spanish-American war, was still well utilized. That the economy of man is well served under such circumstances must remain doubtful.

In this connection it is also necessary to consider psychic or other influences tending to inhibit the development of the appetite secretion; bearing in mind that emotional excitement may destroy the motor (6) as well as the secretory (7) activities of the stomach.

The present paper is a contribution toward determining the relative importance of the various factors involved in the appetite stimulation of gastric secretion, as well as toward estimating the influence of the appetizing or unappetizing character of a meal and of the mental attitude of the subject upon the gastric response and the ultimate digestion of food.

Following the appetite stimulation in each case, the stomach was emptied at regular intervals, using a Rehfuß stomach tube, the volume of secretion determined, its free and total acidity, pepsin and amino-acid nitrogen estimated according to procedures previously described (8).

*The sight psychic secretion and the effect of smell.* Experiments were carried out to determine whether the sight of food alone, or the sight and odor of food combined gave rise to the production of any gastric secretion, as well as to compare the effects on such secretion of the sight of foods prepared in an unpleasing manner.

A breakfast table was set, in a small well-lighted laboratory, with clean linen, bright chinaware, and the following foods were served in a pleasant manner and at one time: ham and eggs, oranges, shredded wheat, bread and butter, hot coffee, cream and sugar. Subjects were brought to this room after any residua had been removed and the level of continuous secretion established. The nose was kept lightly but effectively clamped throughout.

In figure 1 are charted the results obtained with a subject who was a laboratory helper, accustomed to eating lunch at the laboratory. Total volumes of secretion removed are charted with total and free acidities, amino-acid nitrogen values and a so-called total actual acidity which indicates the product of the volume by the total acidity and represents the total amount of acid in the gastric samples.

It will be noted that this subject showed a marked response to the sight of food as regards the volume of gastric juice secreted and the "total actual" acidity. The continuous secretion in this case was high and a greater augmentation by psychic stimulation might perhaps be expected. Under similar conditions another subject (fig. 2) showed a lower and less acid continued secretion. Sight augmented the volume but slightly, although the acidity was distinctly increased.

Somewhat less accustomed to eating in the laboratory were the subjects of the experiments charted in figures 3, 4 and 5, which were carried out in exactly the same way except that clamps were not used. The distinct but not voluminous secretion in these cases may be attributed mainly to the effect of sight as the odor of the meal was not pronounced and odor was found to have little influence on psychic secretion in these subjects. The subjects were, of course, led to believe they would actually receive the food.

To determine whether any elaboration in setting was requisite for the stimulation of secretion by sight, the meal was simplified to a simple half grape fruit served in the usual way. The odor being imperceptible, the noses were not clamped. The results are charted in figures 6 and 7. A stimulation of secretion was brought about by the sight of the food in both cases. It is also probable that some of the psychic secretion may leave the stomach during the intervals of the experiment with the

increase in gastric tonus and produce a stimulation of the pancreatic secretion.

The effect of allowing subjects to seat themselves at a breakfast table prepared in an unpleasing manner was also tried out on six subjects. The same table was used as in the preceding experiments, and the same foods were served. However, the ham and eggs were scorched; the shredded wheat biscuits and bread roughly broken; the coffee and milk weak and diluted; the butter soft; sugar lumpy and dark; the oranges partly squeezed; the dishes generally somewhat greasy and with an appearance of dirtiness induced by the use of charcoal. Newspapers were used in place of linen. The noses of the first two subjects were clamped, of the others free.

The results of these experiments are charted in figures 8, 9, 10, 11, 12 and 13. In only one of these cases (see fig. 12) was any secretion induced above the level of the continuous secretion. It is evident, therefore, that food served in an unpleasant manner will not give rise to an appetite secretion under ordinary conditions, although custom and degree of hunger will naturally influence the conception of an appetizing food.

Breakfasts served in a pleasant manner and with appetizing foods were set before each of the six subjects just mentioned from 15 to 30 minutes after they had been presented with a view of a breakfast of the opposite and discouraging character and which had evoked no psychic gastric response. The results are plotted in the same charts as the preceding tests (figs. 8, 9, 10, 11, 12 and 13).

It will be noted that in the first two cases a marked appetite secretion followed the presentation of the second meal, this being a sight effect as noses of these subjects were clamped. However, the other four subjects did not show a psychic secretion under these conditions. The subjects showing a response were accustomed to eating in the laboratory and may have felt that they would not be expected to actually partake of the disagreeable food. The other subjects having no knowledge of the character of the test might well be more strongly repulsed by the first meal, this effect being carried over for the period of 15 to 30 minutes until the palatable meal was set before them. A secondary effect might also be their suspicion that since they were not permitted to partake of the first meal they might not have a chance at the second, although the contrary view was impressed upon them.

*The psychic secretion and the odor of food.* The influence of the odor of food alone on psychic secretion was tried out on seven subjects (see

figs. 14 to 20). The odor of frying beefsteak was used as a stimulus, the odor being pleasant, strong and unmistakably that of an appetizing food of common consumption. Subjects were blindfolded and the ears were muffled in order to exclude the influence of the sight of the steak and of hearing it fried. Subjects inhaled liberally the fumes arising from the frying steak. As in our other tests, the subjects had had no food for fourteen hours.

Three of the subjects showed no increase in the volume of secretion under the influence of these odors. The four others showed some increase, but in no case was the secretion voluminous. It would appear from these tests that odor was considerably less important than sight in inducing the appetite secretion, at least in man. These results are supported by the experiments previously mentioned, in which combined sight and odor of food brought forth no greater secretion than sight alone. Odor may have an influence on the motor activity of the stomach and may be of importance in animals with a more highly developed sense of smell. There may very well also be considerable differences in individuals of the human species.

*The psychic secretion and the tasting and chewing of food.* The subjects of the preceding tests on the influence of odor were permitted to rest for half an hour to reestablish the level of continued secretion which had in most cases been little affected. They were then permitted to chew for five minutes portions of tenderloin steak with strict caution to swallow none of the pieces, this possibility being checked by careful examinations of the gastric contents. In all cases the subjects remained blindfolded. In the first four cases the noses of the subjects were also clamped so that none of the vapors could be inhaled by that channel. The results are charted in figures 14, 15, 16 and 17, and show no distinct influence of tasting and chewing meat under these conditions upon the secretion of gastric juice. In one case the volume of gastric contents was somewhat increased, but the low acidity shows that very little acid gastric juice could have been secreted. Apparently the taste and chewing of food in the absence of sight or odor produced no marked psychic secretion.

In three other cases the same procedure of chewing and tasting beefsteak was carried out, but the noses of the subjects were unclamped, sight, however, being excluded (see figs. 18, 19 and 20). As illustrated in these cases the influence of the combined tasting, chewing and smelling of food on the secretion of appetite gastric juice was very pronounced and was much greater than that of smell alone.

*The influence of the sound or thought of food.* Subjects were blindfolded and had their noses clamped to exclude the sight and smell of food. They were seated before a frying pan in which a steak was being broiled with plainly audible sputtering and sizzling. They were told what a fine, juicy steak was being prepared for them and a general attempt made to keep their attention on the subject of appetizing meats. The results are charted in figures 21 and 22. In one case the result was negative; in the other case a distinct stimulation of secretion resulted. The variation must be ascribed to individual differences.

After one-half to three-quarters of an hour rest, the nose clips were removed and the subjects permitted to smell as well as hear the sputtering of frying steak. Results are plotted on the same charts and show that in one case a very slight rise in secretion took place. In the other case a definite stimulation occurred, although the earlier level for hearing and thought of food was not surpassed. In one case the subject was permitted to smell feces of a repulsive odor fifteen minutes after smelling steak. Any psychic secretion appears to have been depressed to the level of the continuous secretion but not below this.

Experiment 23 gives a comparison of the psychic effects of: *a*, the sight of a frying steak (ears not stoppered); *b*, sight and smell; and *c*, taste of the same food. A distinct stimulation was produced by the sight of the food. One-half hour later the sight and odor of similar food produced a very similar stimulation. After a further interval of 15 minutes the taste of the food gave a lesser stimulation than sight or sight and smell had previously done.

A summary of some of the results obtained on two of our subjects in so far as volumes and "total actual acidities" of appetite secretions were concerned, is given in figures 24 and 25. They must, of course, be considered in connection with details of individual experiments. They serve, however, to emphasize the important rôle of the sight of food as a stimulus to the appetite.

*The influence of palatability or unpalatability of a meal on its gastric digestion.* Two subjects were given uniform meals prepared and served in the ordinary manner. On a later day they were given the same foods prepared in as unpalatable a manner as possible without altering their chemical composition. The meal used consisted of: cream of wheat, 100 gm.; sugar, 10 gm.; milk, 35 cc.; coffee, 100 cc.; graham crackers, 50 gm.; oranges, 50 gm.; water, 100 cc. On the second experimental day these foods were all mixed together in a conglomerate

mass, discolored with small amounts of burnt crackers and charcoal, and the atmosphere at the table saturated with the repulsive odors of valeric and butyric acids.

The first of these subjects was of a nervous temperament and from his statements and manner was judged to be easily influenced or disturbed by the character and preparation of his food. In fact he positively refused on one occasion to continue eating a meal of the second type mentioned above, although urged to do so in the interest of science. The results on this subject (see figs. 26 and 27) show no delay or inhibition of the acid response of the stomach, although the evacuation time was somewhat prolonged.

The second subject was accustomed to eating in a laboratory, was of a phlegmatic temperament, claiming and appearing to be very little disturbed by the appearance of food or the condition in which it was served. The results on this subject are given in figures 28 and 29. The unpalatable food showed a rapid, though not quite so rapid, development of acidity and a few minutes quicker evacuation.

The first subject was also tried out with a palatable meal 50 minutes after he had violently refused one which he believed to be contaminated. The result is charted in figure 30. The development of acidity was even more rapid than in the case where the meal was given under normal conditions. If any depression of psychic secretion was carried over through this interval, there were no signs of it.

The first subject was also given a meal of unpalatable character similar to the ones already described, but prepared by himself and hence known by him to be innocuous. The results as charted in figure 31 show a rapid development of acidity and quick evacuation.

Some information with regard to the gastric response to foods unpalatable in appearance, odor and taste was obtained by experiments on the feeding of Chinese preserved duck eggs called "pidan." These eggs have dark greenish yolks and yellow-brown "whites" of a firm, gelatinous consistency and possess distinct odors of ammonia and hydrogen sulphide. One subject disliked these eggs but did not know what they were and was not especially prejudiced against them. The other subject, "Don," was of a nervous type, and just as he finished eating the eggs he was told in a joking manner by one of the laboratory wits that they were of prehistoric Chinese origin. The subject became clearly suspicious that something had been given him that was not entirely fresh. The results of this test as compared with similar tests on boiled duck eggs and on raw white and yolk of egg are given in

figure 32. They show a depression of gastric secretion after "pidan" lasting for an hour and a quarter, the acidity then rising rapidly to normal figures. This delay may have been due to inhibition of appetite secretion, gastric activity being finally aroused through chemical stimulation following the solvent action of the slow continued secretion.

The failure of this unappetizing food to arouse the secretory or motor activities of the stomach to a normal response is indicated also by our results on the first subject mentioned above. The curves are given in figure 33 and show that while raw hens' eggs gave an acid response of over 100 in the first hour and left in  $2\frac{1}{4}$  hours, the preserved eggs at no time gave acidities of over 30 and remained in the stomach  $4\frac{1}{2}$  hours. It appears that the unappetizing character of these eggs led to a delayed acid response and slow evacuation, perhaps complicated by their failure to show some early digestion with consequent chemical stimulation.

*Influence of prejudice against a food on its digestibility in the stomach.* It is very common to find people who have a prejudice against certain foods generally classed among the most wholesome articles of diet. Certain cases may be due to a food anaphylaxis or sensitivity. Others may be due to defective gastric or intestinal digestion or other causes. Undoubtedly many have no foundation and are the results of wrongly placing the blame of certain digestive disturbances.

One of our subjects, "Ham," had a strong prejudice against eggs in any form and had not eaten them for years. He was with difficulty persuaded to take eggs prepared in several different ways. The results of these tests are plotted in figure 34, and show that eggs were digested by this subject in a perfectly normal manner, at least as far as the stomach was concerned. Neither did untoward symptoms of any kind develop.

*Influence of newspaper reading on gastric digestion.* Subjects were permitted to read newspapers throughout the course of a meal of palatable foods, the same test meal as used in previous experiments. The gastric responses of two subjects who read newspapers and responses of same subjects with no reading but with usual table talk are charted in figures 26, 28, 35 and 36. No distinct influence of newspaper reading was noted. Responses were quite normal in all cases. The slight differences in acid development and evacuation time were in one case favorable and in the other case unfavorable to newspaper reading.

*Influence of the unpalatable character of a diet on its ultimate utilization by the human body.* Smith, Holder and Hawk found (9) in a metabolism experiment on a normal man that where a uniform diet of a



palatable character was given for several days, followed by a period in which the same foods were jumbled together in dirty dishes and served amidst ill-smelling and otherwise unpleasant surroundings, that the nitrogen utilization in the first case was 86.7 per cent and in the second, 85.6 per cent. The nitrogen balance showed a retention in the first period of 3.0 per cent and in the second, of 6.4 per cent. This in spite of the fact that the subject was only with difficulty persuaded to eat the unpalatable food and that another subject who was given the same kind of food became nauseated and could not continue.

*Influence of anxiety on gastric digestion* (10). The study of the influence of emotional strain on digestion in man offers some difficulties due to the fact that the emotions cannot be readily controlled, nor are the subjects of extreme emotion readily amenable to experimentation. We were, however, able to obtain an interesting illustration of the profound effect of mental anxiety on gastric digestion in the case of one of our subjects. The man was a first-year medical student who had previously served as a subject of gastric tests. He was given 100 grams of fried chicken on the morning of an important examination in chemistry and was asked to write out his answers during the course of the test. He was plainly worried over the outcome of the examination and of his year's work. The effect upon gastric digestion was the prolonging of the evacuation time to  $6\frac{1}{2}$  hours. The intra-gastric acidity remained in the neighborhood of 90 for 3 hours. The normal digestion curve for fried chicken on this subject was obtained a week later under the best mental conditions. The time required was  $4\frac{1}{2}$  hours and the maximum acidity about 65. It is not at all surprising that worry aggravates a condition of gastric ulcer.

An interesting experiment on the digestion of milk in the human stomach may be cited in this connection (11). It was found that in the stomach of one of our subjects milk would not curdle. The test was carried out at the end of the year immediately before the final examinations. The subject was one of the most brilliant students in his class and had worked hard. We made several tests on this student and in every case milk left his stomach rapidly and without curdling. He digested all other food normally. The next fall, upon his return to college, we made another milk test upon him and found that his stomach curdled milk in a normal manner. At this time he was in fine physical condition, having had a long, pleasant vacation, whereas in the spring he was in a highly nervous state as a result of his hard study. This serves to illustrate the influence which rigid and prolonged mental application may exert upon the stomach in certain types of individuals.

## SUMMARY AND CONCLUSIONS

The sight alone of a table well set with nourishing foods was found to give rise to a distinct secretion of gastric juice in normal men. The sight of a half grape fruit only resulted likewise in an appetite secretion. The sight of the same foods illy prepared and poorly served resulted in no stimulation of appetite secretion. The service of a well prepared meal half an hour after the service of a poorly prepared one gave in some instances a distinct secretion, in others not.

The odor alone of frying meat produced in some cases no appetite secretion, in others a slight secretion. Odor alone produced less stimulation than sight alone.

The tasting and chewing of food in the absence of smell or sight produced no marked psychic secretion. The combined influence of the tasting, chewing and smelling of food was pronounced and much greater than that of smell alone.

The sound and thought alone of a frying steak gave rise to a gastric secretion. The influence of smell with hearing produced little additional effect. Evil odors depressed secretion to the level of the continuous secretion.

In consecutive tests the sight of food, with and without odor, produced similar degrees of stimulation, while taste alone had less effect.

Mixed meals consisting of nourishing ingredients but very unpleasantly prepared and served gave rise in the case of a phlegmatic individual to no distinct delay in the development of intra-gastric acidity or in evacuation. A more susceptible individual showed a slight delay in evacuation time, but none in acid response.

Chinese preserved eggs, unpalatable to our subjects in appearance, odor, taste and belief in their unwholesome character led to delayed acid response and evacuation. In one case the normal acid level was ultimately attained due to chemical stimulation.

In one subject a strong prejudice against eggs was found not to result in any abnormal gastric response when eggs were eaten.

The ultimate utilization of the protein of a diet prepared in a most unpalatable manner was not found to be appreciably less than that of the same diet served under the best conditions.

Newspaper reading during the course of a meal could not be shown to have any distinct influence on gastric digestion.

Anxiety and mental strain were found to markedly delay gastric digestion.

The authors desire to thank for their coöperation the students of Jefferson Medical College who acted as subjects of these tests.

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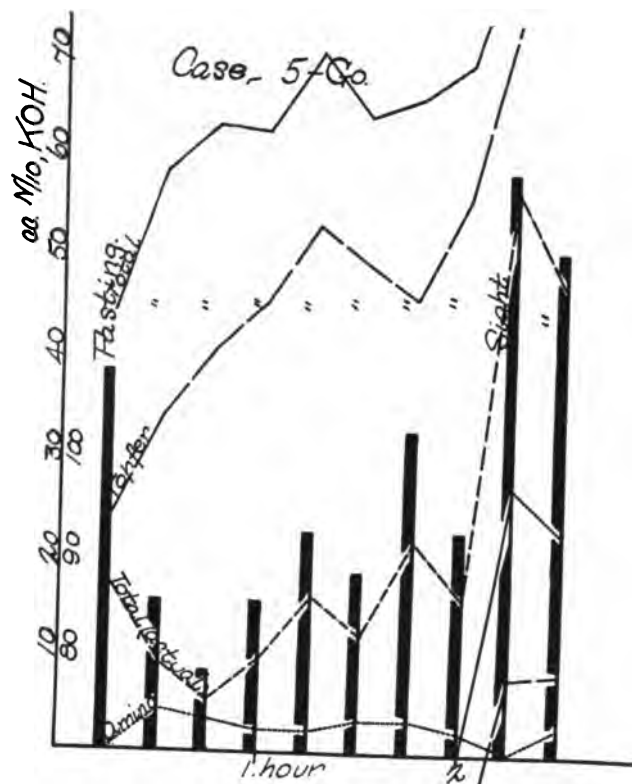


FIG. 1.

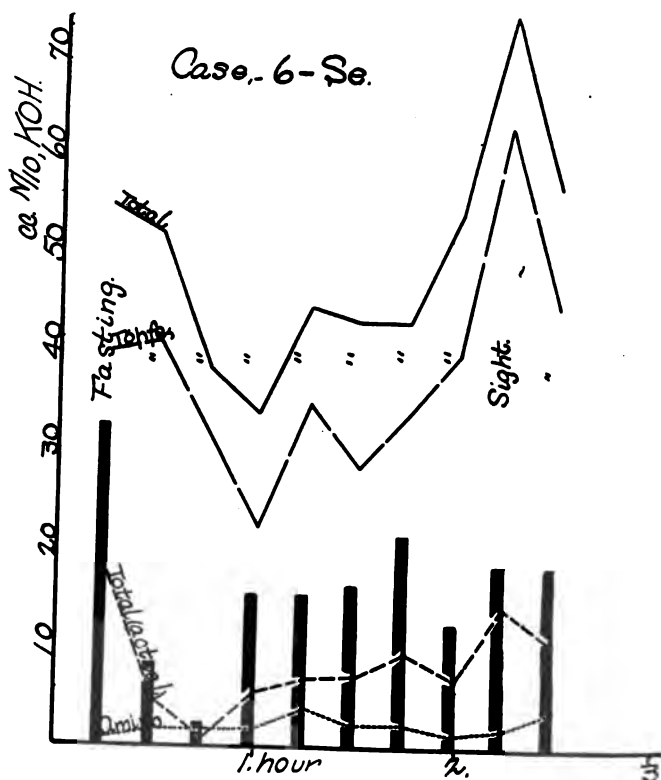


FIG. 2.

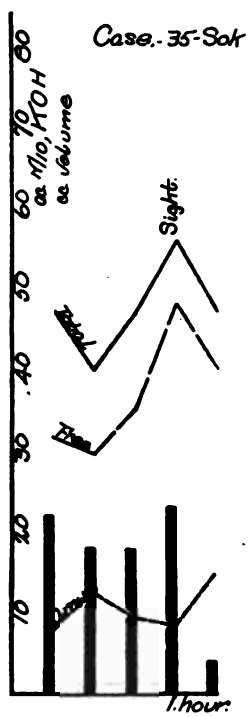


FIG. 3.

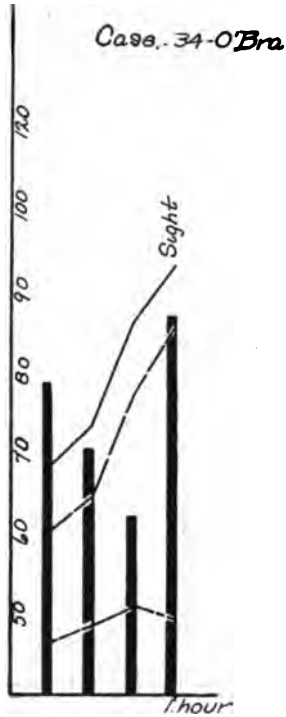


FIG. 4.

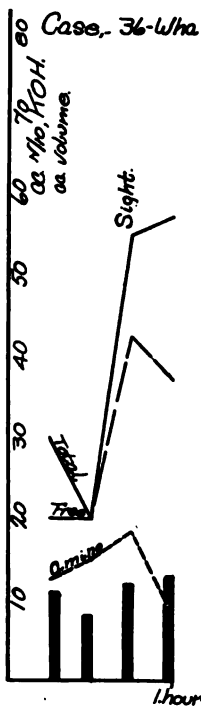


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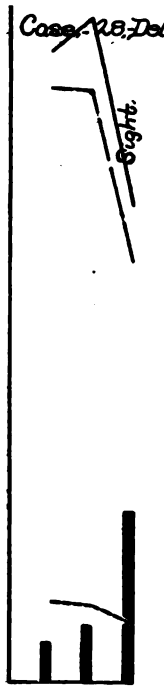


FIG. 6.

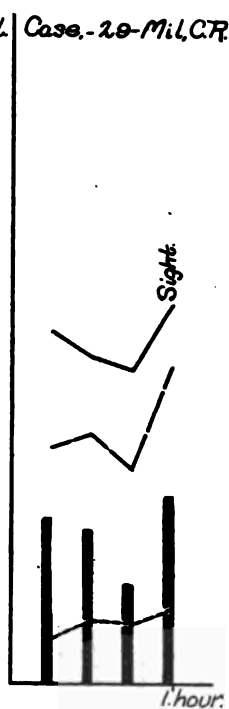


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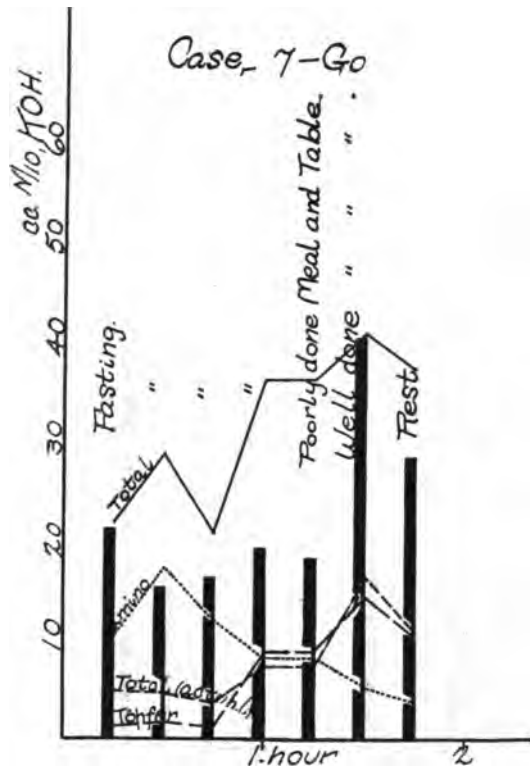


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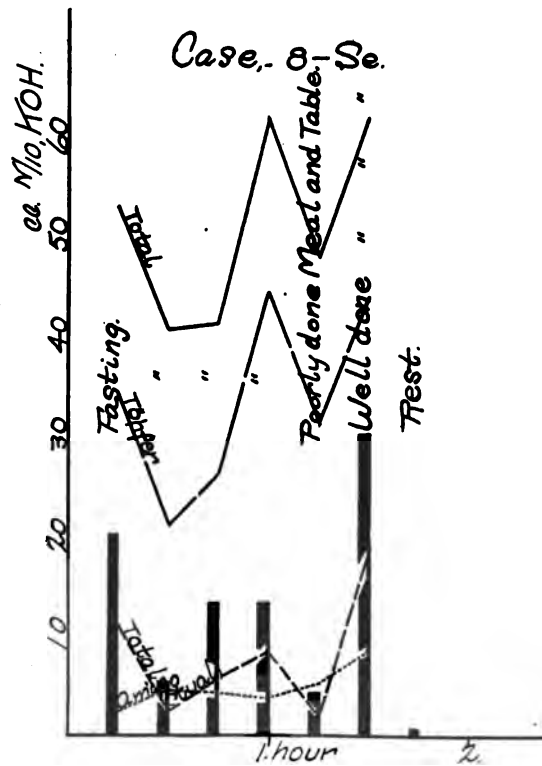


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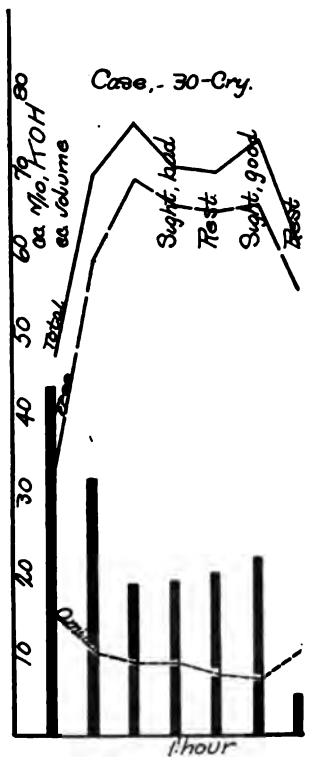


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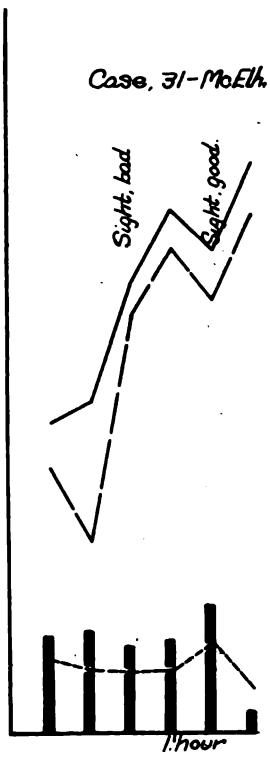


FIG. 11.

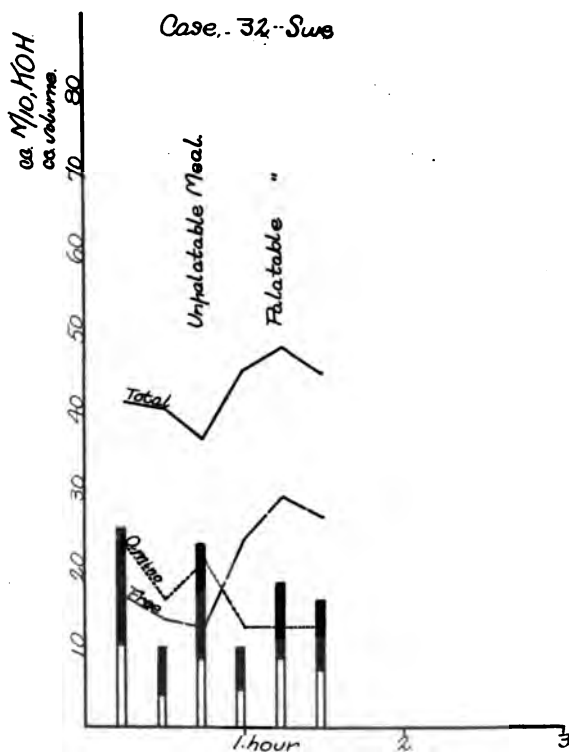


FIG. 12.

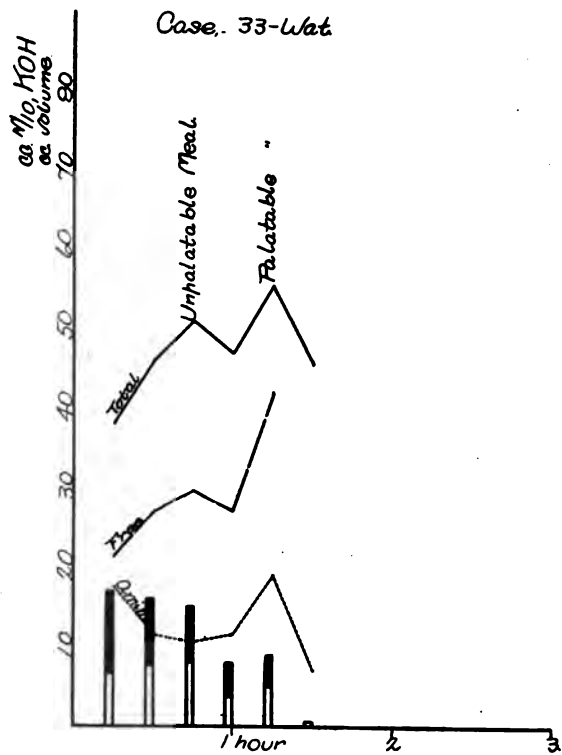


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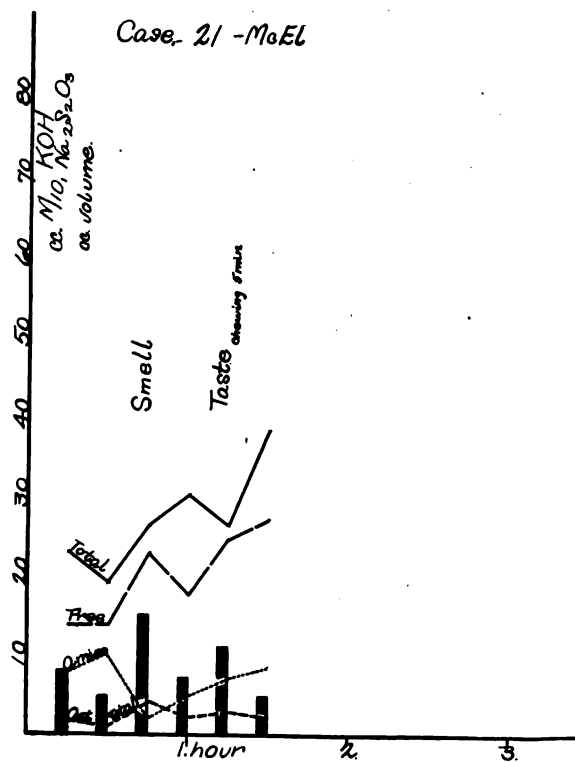


FIG. 14.



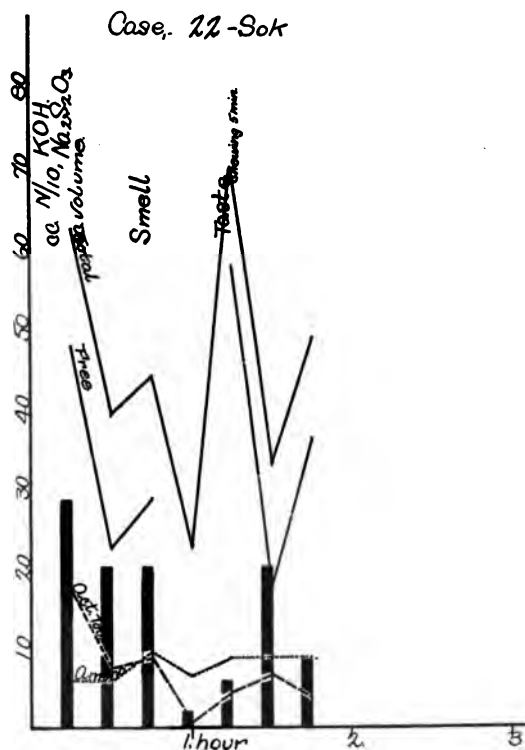


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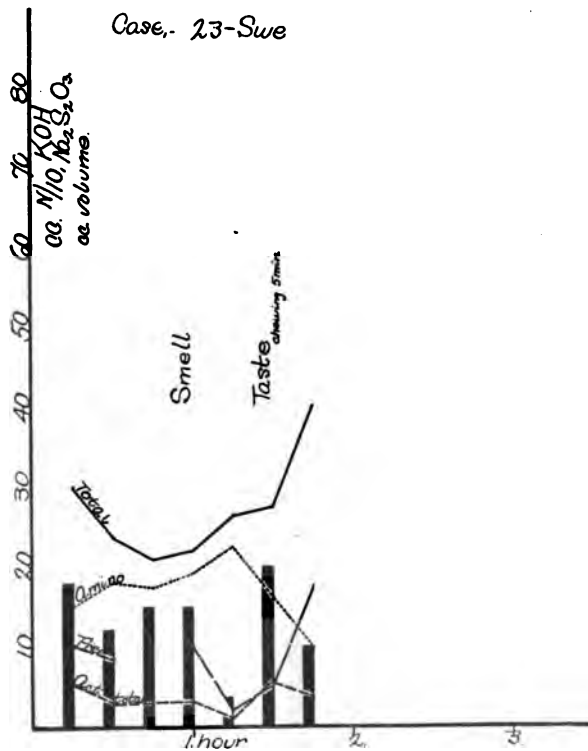


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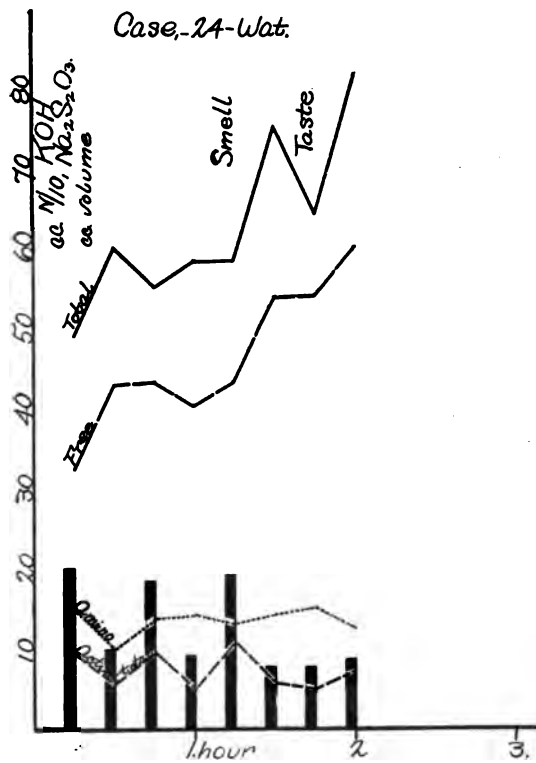


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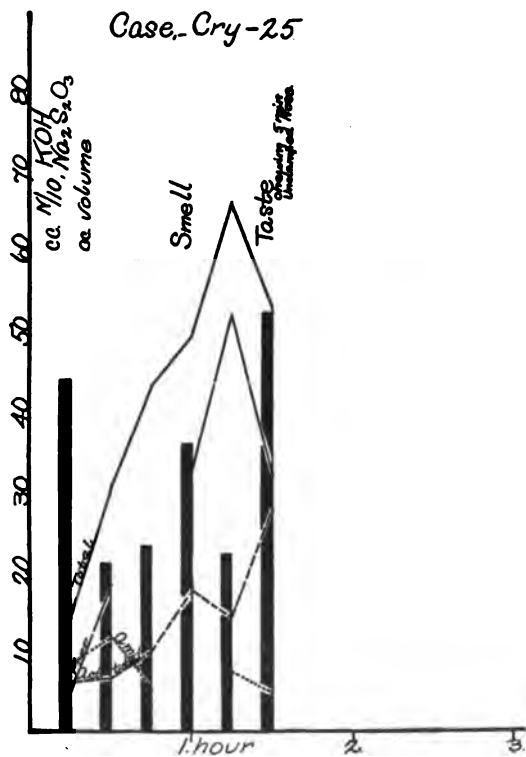


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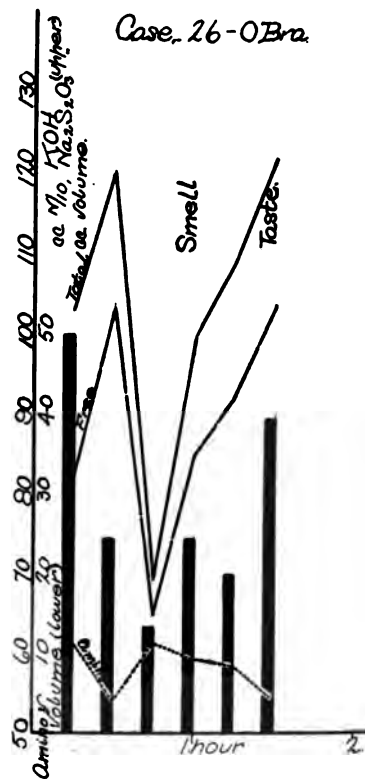


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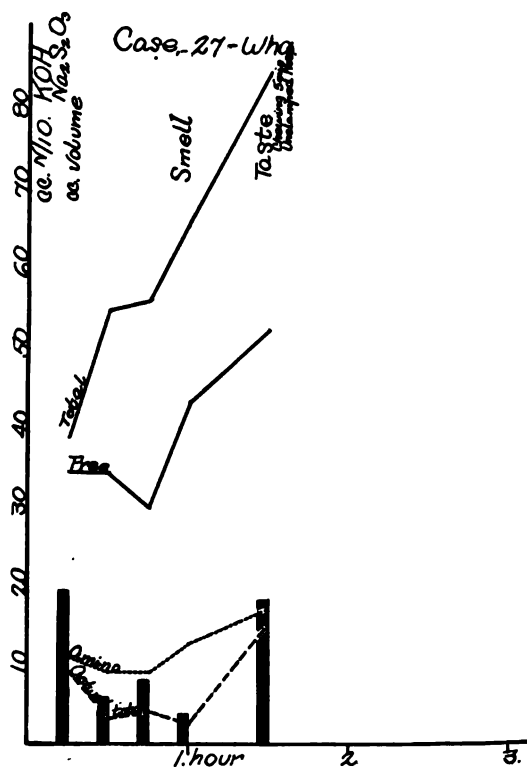


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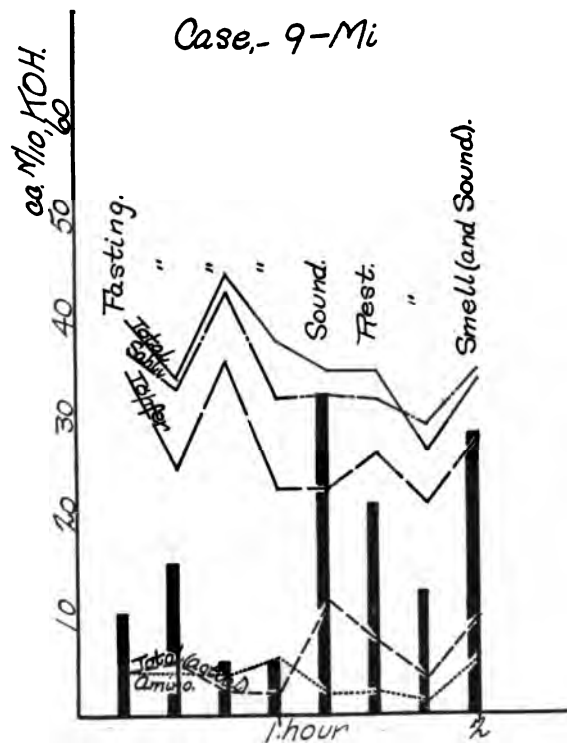


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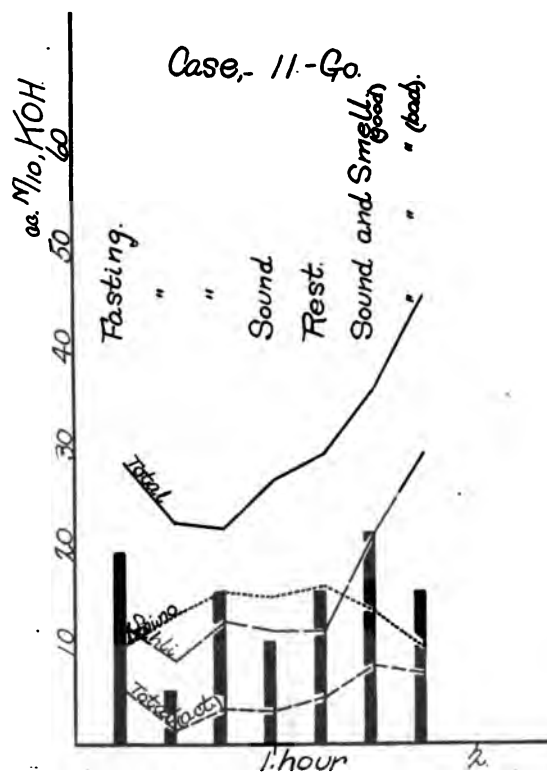


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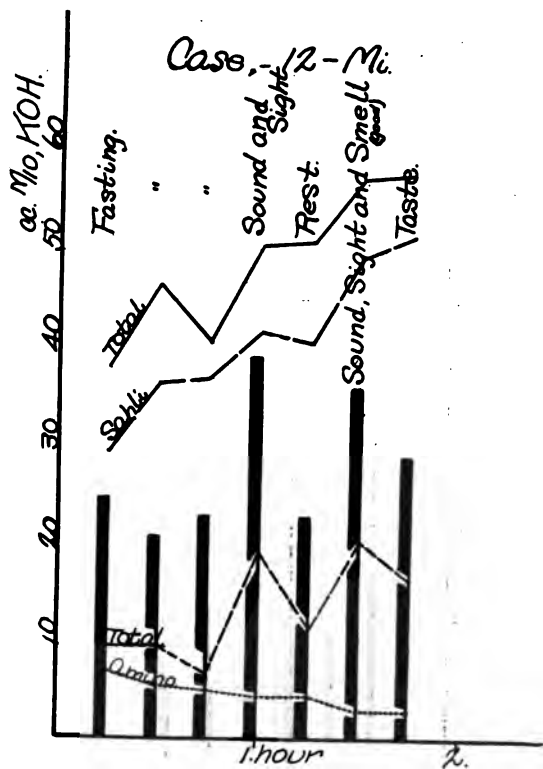


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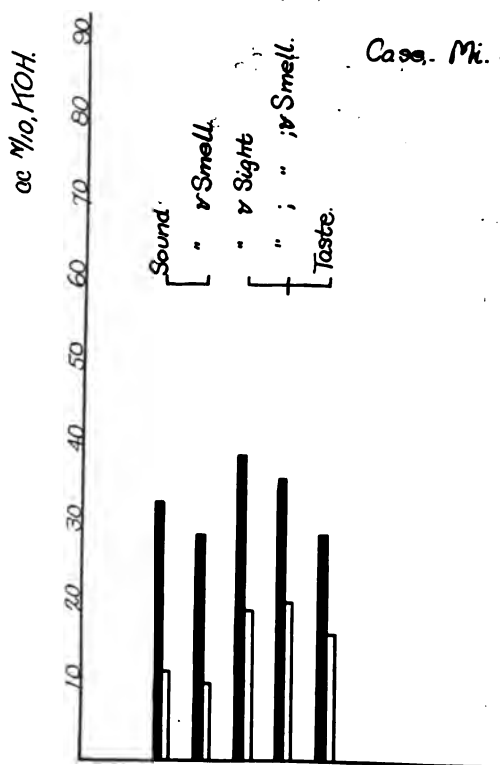


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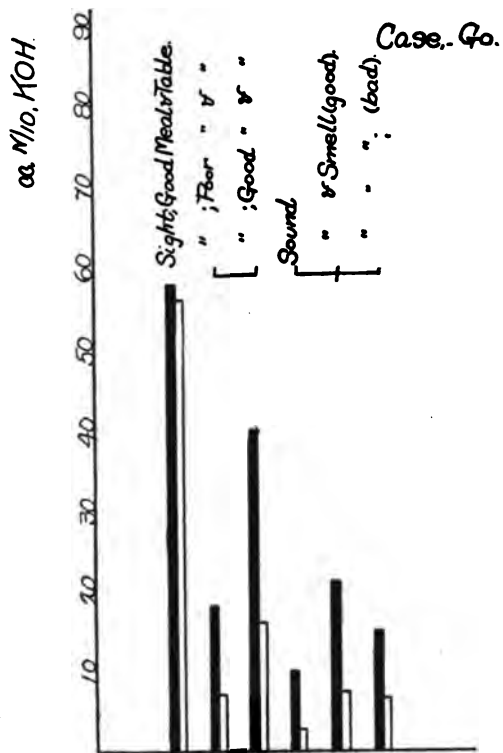


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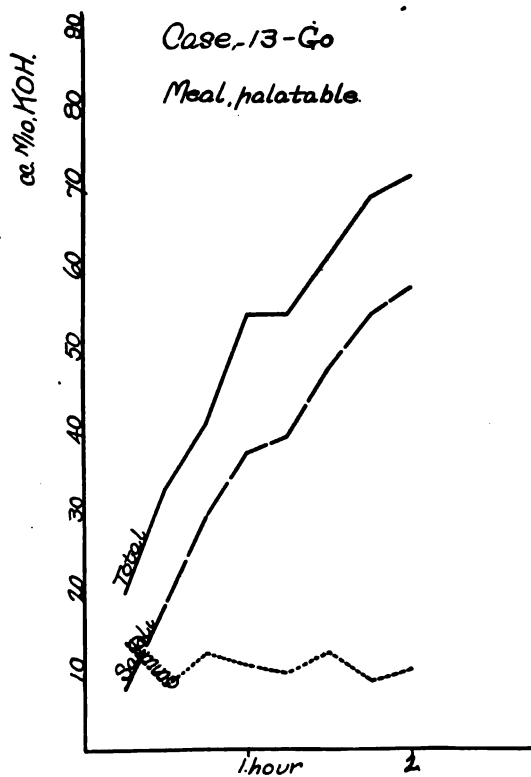


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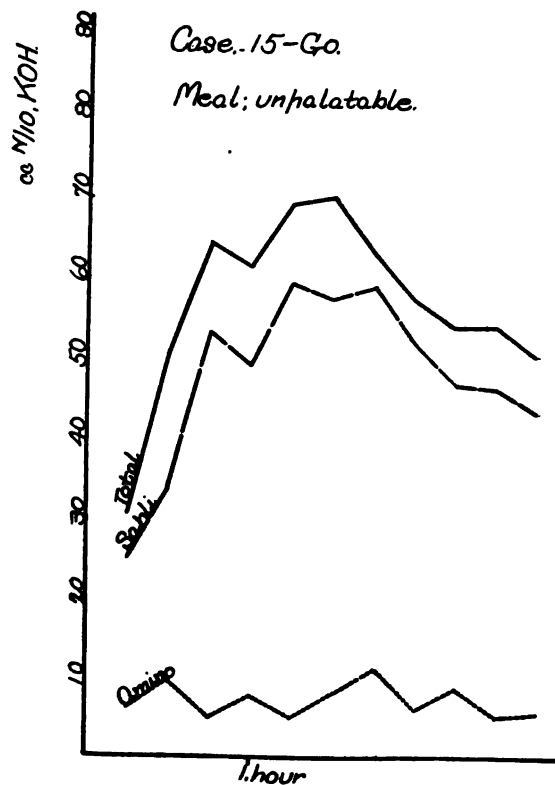


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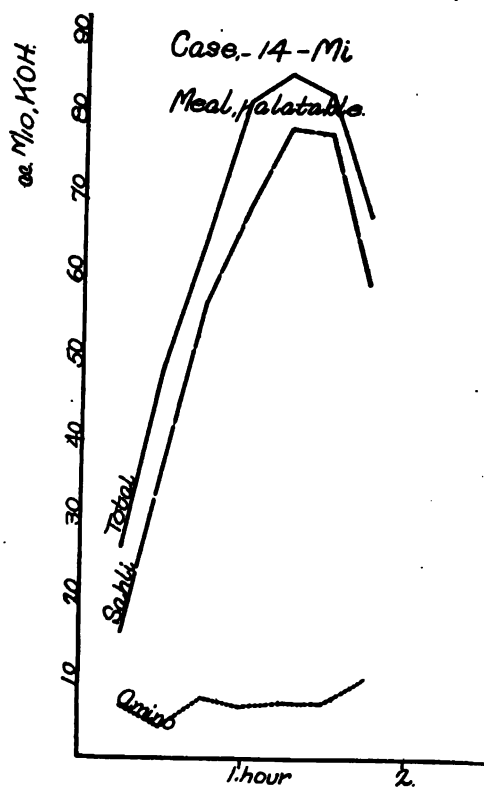


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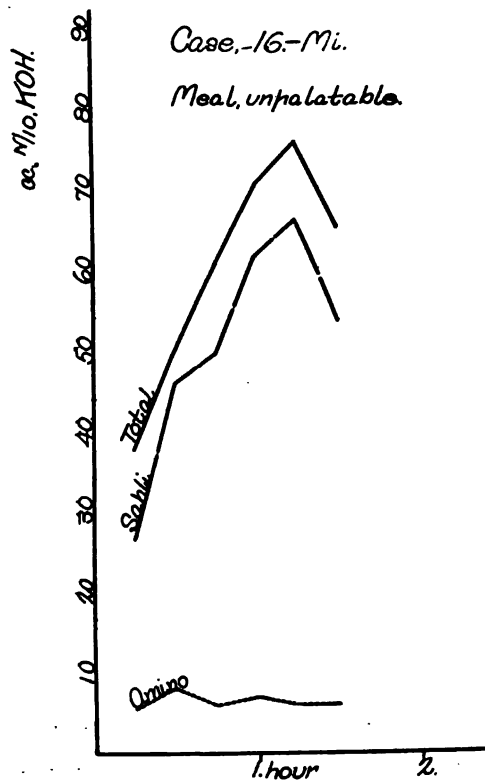


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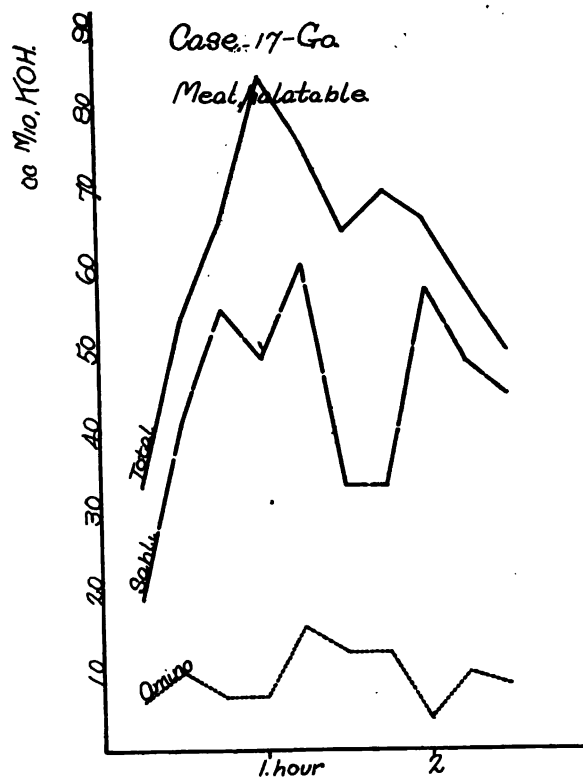


FIG. 30.



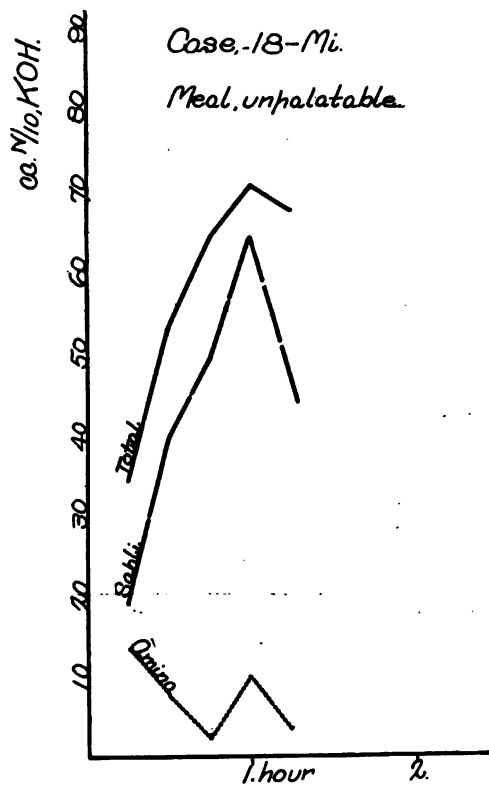


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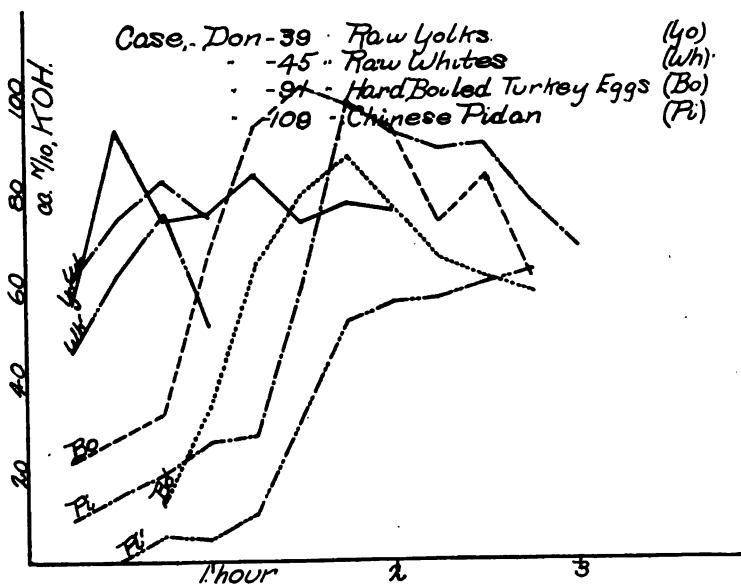


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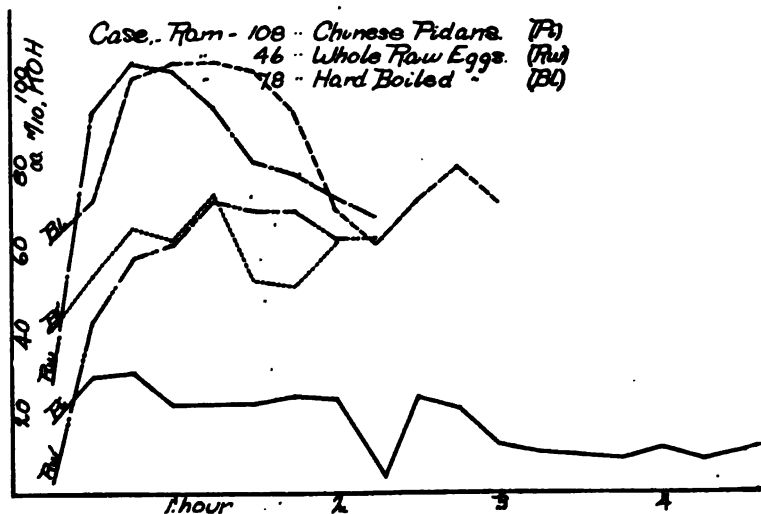


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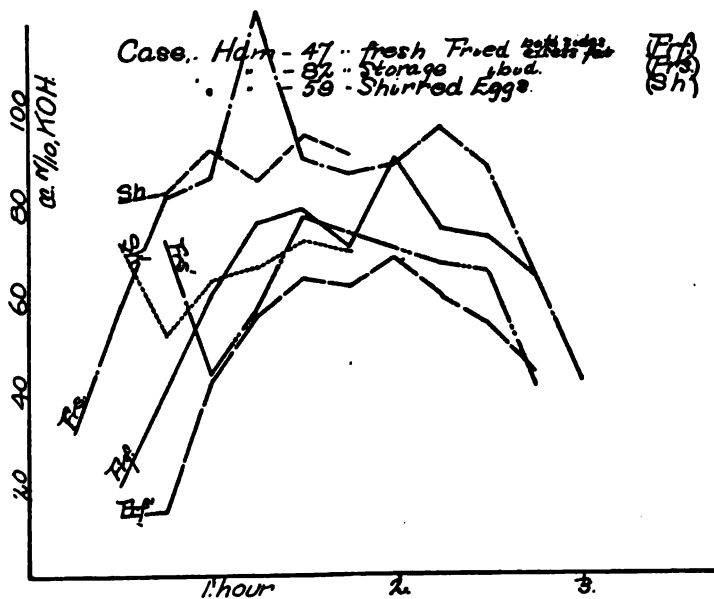


FIG. 34.

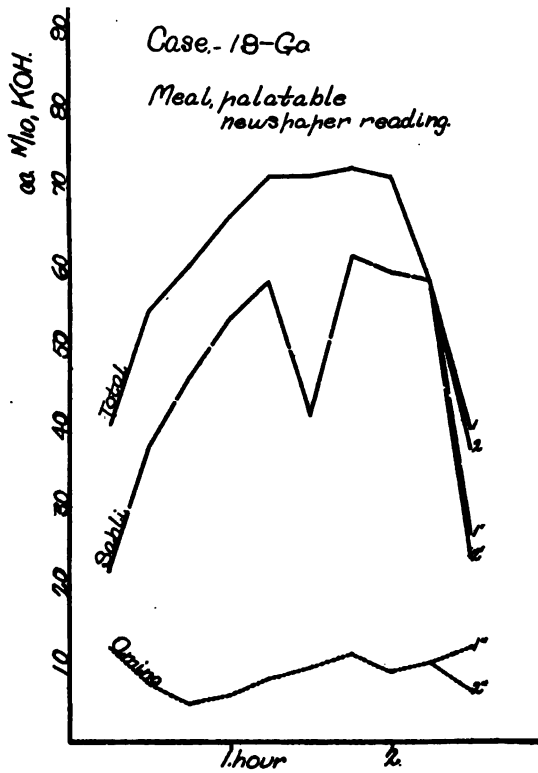


FIG. 35.

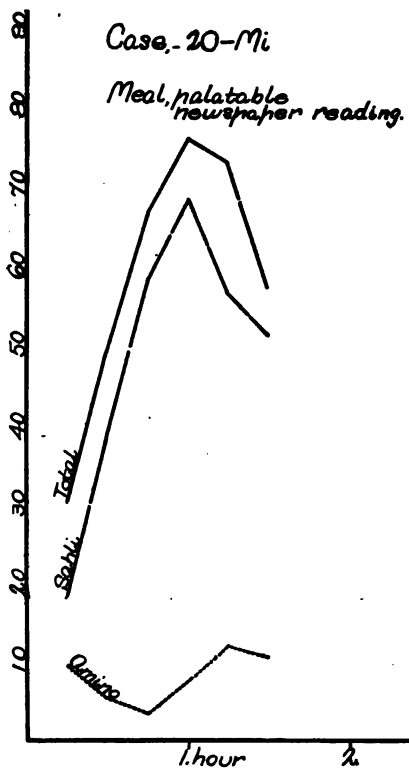


FIG. 36.

## GASTRIC RESPONSE TO FOODS<sup>1</sup>

### XI. THE INFLUENCE OF TEA, COFFEE AND COCOA UPON DIGESTION

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Many experiments have been made to determine the effects of tea and coffee on peptic digestion in vitro. The object of the series of experiments reported here was somewhat different, namely, to determine in what way the responses of the stomachs of normal human subjects to mixed meals would be affected by adding to such meals equal volumes of water, tea, coffee or cocoa—hot or cold—and with or without the addition of cream or sugar. Certain observations relative to changes in pulse rate and other symptoms following ingestion of these beverages were also made, but the experiments were not complete in this respect.

We are not familiar with any previous experiments of the type carried out by us. Penzoldt (1), however, studied the evacuation of 200 cc. portions of water, tea, coffee and cocoa on a single subject and found them to leave the stomach as follows: Water, 1 hour, 15 minutes; tea, 1 hour, 30 minutes; black coffee, 1 hour, 45 minutes; coffee with 5 cc. cream, 2 hours, 15 minutes; cocoa with water, 1 hour, 45 minutes; cocoa with milk, 2 hours, 30 minutes.

Our experiments were carried out upon normal medical students. They reported at 8:00 a.m. without breakfast, and without removal of residuum they were given the following uniform meal, either alone or with the beverage to be tested which was drunk during the course of ingestion of the other foods.

*Test meal:* Scraped beef, grilled, 75 grams; mashed potatoes, 50 grams; toast, 40 grams; butter, 15 grams; salt, 1½ to 2 grams as desired.

The tea, coffee and cocoa used were of good quality and prepared of a moderate strength by the dietitian of the hospital, exactly as used in the regular dietary of the institution. Where sugar was used, four

<sup>1</sup> The expenses of this investigation were defrayed by grants from Mrs. M. H. Henderson, The Curtis Publishing Company and Dr. L. M. Halsey.

lumps of sugar per liter were given. In the case of cream approximately 15 cc. of a 20 per cent cream were used. No milk or added sugar were used in preparing the cocoa, which was made up according to the directions on the container.

As soon as the meal was finished the subjects were made to swallow the Rehfuß stomach tubes. From 5 to 8 cc. of the stomach contents were aspirated at 15-minute intervals for  $1\frac{1}{2}$  hours. The subjects were then allowed to attend a class and returned  $1\frac{1}{2}$  hours later (after a total experimental period of 3 hours). Then two samples were withdrawn at 10-minute intervals, and finally the stomach was completely emptied, this being checked by a lavage of 150 cc. of water.

Total and free acidities were determined by titration and pepsin by the Mett method. The volumes of contents at the end of the 3-hour period were measured. The pulse rate was also determined at intervals and subjects reported any unusual symptoms.

Tests were carried out in which the following beverages were added to the uniform test meals:

- (1) Cold water, 1 liter, 15°C.
- (2) Hot coffee, plain, 1 liter, 45–50°C.
- (3) Hot coffee, with sugar, 1 liter, 45–50°C.
- (4) Hot coffee, with cream, 1 liter, 45–50°C.
- (5) Hot coffee, cream and sugar,  $\frac{1}{2}$  liter, 45–50°C.
- (6) Cold tea, plain, 1 liter, 13°C.
- (7) Hot tea, plain, 1 liter, 50°C.
- (8) Hot cocoa, plain, 1 liter, 57°C.
- (9) Hot cocoa, plain,  $\frac{1}{2}$  liter, 57°C.

Thirty-seven experiments were carried out on four different subjects, duplicates being made on each subject with the basal diet alone. The results are charted in figures 1 to 34. The results with regard to evacuation time are summarized in figure 34.

#### INFLUENCE OF BEVERAGES ON THE ACID RESPONSE OF THE STOMACH

*Influence of cold water on the acid response.* The diet alone containing, as it does, considerable meat gives rise to the rapid development of a relatively high acidity, much of which is represented by combined acid (figs. 1, 10, 19 and 26). As no liquid whatever was taken with the meals in these cases and in the early part of digestion the absorption of gastric juice by the meat was pronounced, a uniform mixture

was not at once obtained in the stomach; and the samples withdrawn varied somewhat with the exact location of the tip in the stomach. For this reason the duplicate curves do not coincide, in spite of the fact that digestion follows the same course and is concluded after the same lapse of time. Where a moderate amount of liquid is present or the combining power of the food less, it is possible to sample uniformly and to obtain very similar curves with the same food eaten at different times. For this reason the experiments on different beverages are, with regard to acid response, more comparable with each other than with the diet lacking liquid.

The curves of acidity, especially total and combined acidity, appeared to rise somewhat more quickly and to a generally higher level with the diet alone than with the added liter of cold water. As the evacuation time was practically unaffected and the acidities still high following the high water ingestion, the water cannot be said to have hindered digestion and must have given rise to a considerable stimulation of acid secretion or been rapidly emptied in large measure, or more probably both.

In consideration of the results of these tests it must be constantly borne in mind that unusual amounts of the various beverages were given. This was intentionally done in order that any untoward influences might be accentuated.

*Influence of coffee on the acid response.* Hot coffee, plain, that is without cream or sugar and at a temperature of 45 to 50°C. was given to four subjects. The acid responses are charted in figures 3, 12, 21 and 27. In the first three cases a liter of the coffee was taken; in case 27, however, only half a liter. The charts show in a general way that the acid responses were very similar to the results obtained with cold water, and not very different from those obtained with the basic meal by itself, although not attaining quite the heights of total acidity given by the latter. There was no distinct indication that the coffee inhibited the secretion of the gastric juice.

Sugar was added to the coffee in two cases (see figs. 4 and 22). Comparisons of the acid responses with those of the same individuals taking plain coffee (see figs. 3 and 21) show that the sugar depressed considerably the acid response. It has been clearly demonstrated in this laboratory that sugar depresses gastric secretion; and it is, therefore, improbable that the low acid values in the earlier period of digestion in the cases where sugar was added to the coffee, are due merely to the slower evacuation of the beverage.

The addition of moderate amounts of cream (15 cc. of 20 per cent cream to the liter) was tried out in two cases (compare figs. 12 and 13 and figs. 27 and 28). It will be noted that the acid response was not demonstrably influenced by such addition.

Hot coffee with both cream and sugar was given in four cases (see figs. 5, 14 and 29). The results indicate that with the addition of cream and sugar the acid response was higher than where sugar alone was used, and more nearly approached that of the plain coffee. This may perhaps be explained by the fact that the coffee was more appetizing with additions of cream and sugar, provoking a more marked appetite secretion and also leading to more rapid evacuation.

*Influence of tea on the acid response.* One liter of cold tea (12° to 13°C.) was given during the course of the standard meal to each of these subjects (see figs. 6, 15 and 30). If the acid responses are compared with those of the same subjects to cold water or plain hot coffee, it will be noted that they are very similar, although one subject showed a slightly higher curve with tea than with water or coffee.

Hot tea (50°C.) was also given to three subjects (see figs. 7, 16, 24 and 31). With slight variations, generally in favor of the hot tea, the results obtained were not distinctly different from those with water, coffee or cold tea.

*Influence of cocoa on the acid response.* That cocoa when taken in amounts of 1 liter at a meal has a depressing action on the development of gastric acidity is clearly apparent from the results on our four subjects as charted in figures 8, 17, 25 and 32. In each case the intragastric acidity rises slowly as compared with either cold water, hot coffee or hot or cold tea. This may be due to several causes. Undoubtedly the sugar content depresses secretion and delays evacuation as sugar solutions have been clearly shown to do. The higher fat content of cocoa may play a part as well as the presence of other constituents.

In three cases the amount of cocoa drunk was reduced to one-half liter. The depressing action of cocoa on the development of acidity may be noted in these cases (see figs. 9, 18 and 33). In general this depression is about equal to that of one liter of tea or coffee.

#### INFLUENCE OF WATER, TEA, COFFEE AND COCOA ON THE EVACUATION TIME OF THE NORMAL HUMAN STOMACH

The influence of the beverages studied on the evacuation time of the stomach is summarized in figure 34, which gives the volumes of the gastric contents at the time of complete removal (approximately 3 hours after ingestion). The evacuation of the stomach was not appreciably delayed by the drinking during the meal of 1 liter of cold water; hot coffee, plain; hot coffee with cream; hot coffee with cream and sugar; cold tea, or hot tea; nor were there any distinct differences between the evacuation periods of any of these beverages. The addition of sugar alone to coffee did, however, delay evacuation distinctly in both cases. Most striking, however, was the pronounced delay in evacuation caused by cocoa, this being noted in every case, even where half amounts only were given. For example, in one case the gastric contents at three and one-half hours measured, following the meal alone, 124 cc.; following hot coffee with cream and sugar, 110 cc.; and after cocoa, 465 cc.

Temperature appeared to have little influence on evacuation time, meals including cold tea leaving the stomach in the same time as those including equal volumes of hot tea; nor did cold water delay evacuation.

#### INFLUENCE OF TEA, COFFEE AND COCOA ON THE PEPTIC RESPONSE

Peptic activities were determined by the Mett method. In figure 35 are illustrated the values obtained after the uniform meals had been given with water, tea, coffee and cocoa respectively. The only striking differences in the development of peptic activity noted were the low values found after the ingestion of coffee with sugar and especially after the ingestion of cocoa. These low values may be due to the depression of the secretory mechanism by the sugar and other substances present in cocoa as well as to retention of the ingested beverages.

#### INFLUENCE OF TEA, COFFEE AND COCOA ON THE PULSE RATE

The pulse rate was also followed in each case, although not as systematically as would be necessary for exact comparisons of the effects of the different beverages upon the heart beat. Tea, and particularly coffee, did, however, bring about in liter quantities a pronounced acceleration of the heart beat in the first half-hour, one subject showing an



increase to 150 and 160 beats per minute after hot tea and coffee respectively. There was some evidence that coffee brought about a more rapid and pronounced acceleration of the heart beat than the other beverages. From its slow evacuation, cocoa might be expected to produce a less rapid stimulation. An attempt (2) has been made to account for the more rapid action of coffee as compared with tea by showing that in coffee the alkaloid exists as caffeotannic acid soluble in cold water and not readily precipitated, while tea contains a caffeine tannate soluble in hot but not cold water and which is readily precipitated by acid (hence presumably by the gastric juice), going readily back in solution in an alkaline medium (such as that of the pancreatic juice). While coffee is usually about a 6 per cent decoction and tea about  $1\frac{1}{2}$  per cent, it must be borne in mind that tea contains 3 to 4 per cent of caffeine and coffee about 1 per cent.

*Other systemic effects of tea, coffee and cocoa.* Tea and coffee in the amounts taken gave rise in our subjects to nervousness, vasomotor relaxation, sweating, tremors, headaches, dizziness and sleeplessness, in some cases to a marked degree. Three of our subjects were unaccustomed to the use of coffee, and the fourth never drank more than one cup at a meal. It is, therefore, probable that the symptoms noted were of a somewhat more aggravated character than would be found in the case of persons habitually drinking much tea or coffee. There is no question, however, that tea and coffee may have a marked effect upon the circulation, and that they are in no wise to be considered as beverages to be used in an unrestricted manner.

Cocoa did not give rise to nervous or vasomotor symptoms to anything like the same extent as tea and coffee. This may in part have been due to slow absorption. In all cases there was a feeling of fulness after cocoa and a lack of hunger, which may be readily explained by the prolonged retention of this beverage in the stomach.

#### DIURESIS AFTER TEA AND COFFEE DRINKING

The urine secretion of our subjects was measured during the period immediately following the ingestion of the test meals with tea, coffee and cocoa. The volumes of urine eliminated are indicated by charts 36, 37 and 38. As much as 866 cc. of urine was excreted within an hour and a half after 1 liter of tea was given, and the values after coffee drinking were of the same order. It is clear, therefore, that these beverages left the stomach quickly, were rapidly absorbed, and the excess

water soon eliminated. Coffee with sugar resulted in less rapid elimination, and the secretion after cocoa was low in volume, as might be expected from the delayed gastric evacuation. The specific gravities of the urines during the period of most rapid elimination varied from 1.004 to 1.001 or less, indicating a very dilute secretion.

#### SUMMARY AND CONCLUSIONS

A study was made of the influence of water, tea, coffee and cocoa upon the gastric digestion of a uniform mixed meal as measured by the acid responses and evacuation times.

Evacuation of the stomach was not appreciably delayed by the drinking of 1 liter of cold water, cold or hot tea, hot coffee, either plain, with cream or with cream and sugar. The addition of sugar alone to coffee delayed evacuation.

Cocoa in 1 liter quantities markedly delayed evacuation. To a less extent this was true of half-liter volumes.

One liter quantities of water, hot or cold tea, hot coffee, plain or with cream, delayed somewhat the rise of the level of intragastric acidity as compared with the basal meal alone. As high acidities and normal evacuation were, however, attained these beverages must have stimulated gastric secretion, been rapidly evacuated, or more probably both.

Coffee with sugar alone delayed the development of gastric acidity. Coffee with sugar and cream had less effect.

Cocoa delayed distinctly the development of intragastric acidity.

One liter quantities of tea and coffee gave rise to marked acceleration of the heart beat, to vasomotor relaxation, tremors and other nervous symptoms.

Cocoa did not produce these effects but brought about a feeling of fulness at the stomach.

Urine secretion during the first 90 minutes after tea or coffee ingestion varied from 550 to 866 cc., after cocoa from 125 to 372 cc.

The authors desire to thank the students who kindly served as the subjects of these tests.

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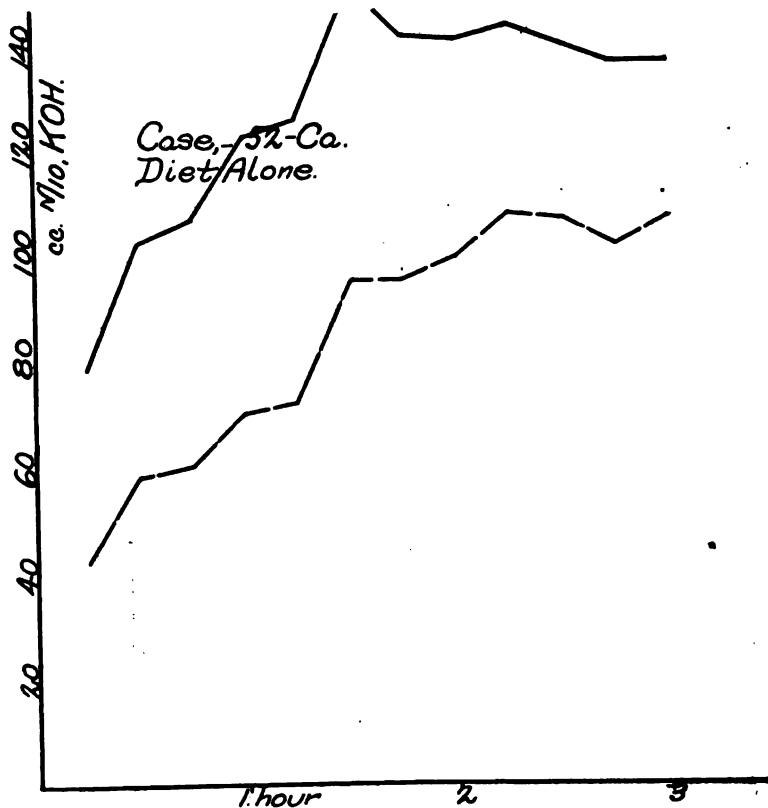


FIG. 1

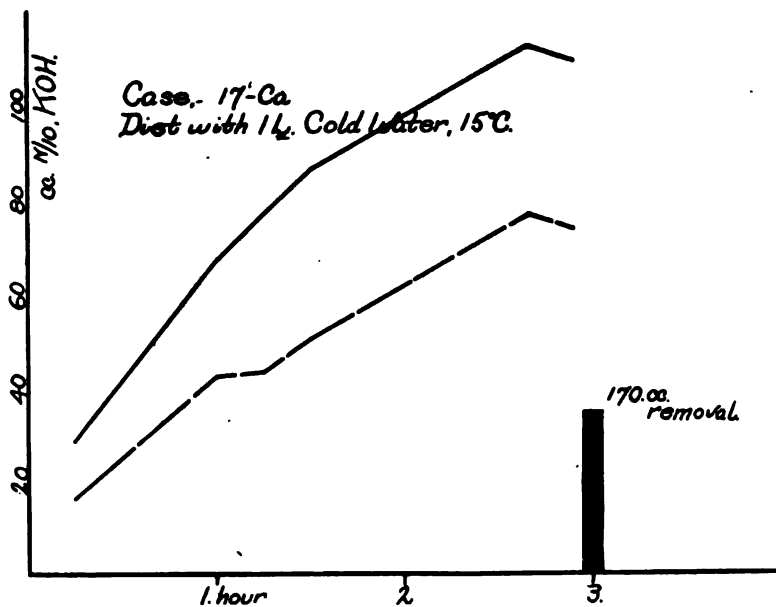


FIG. 2

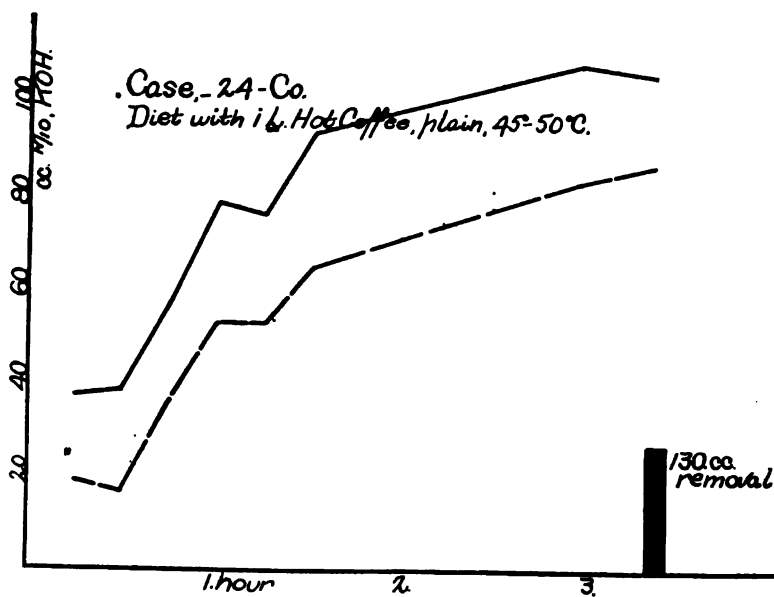


FIG. 3

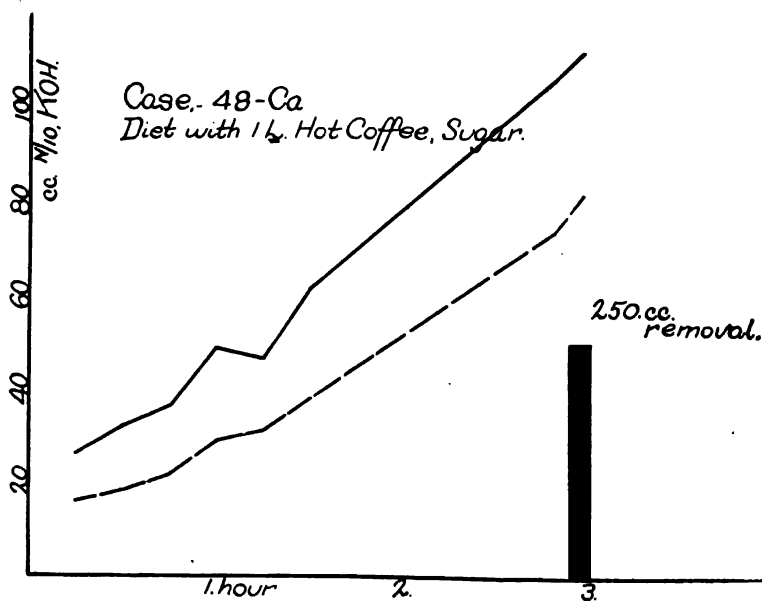


FIG. 4

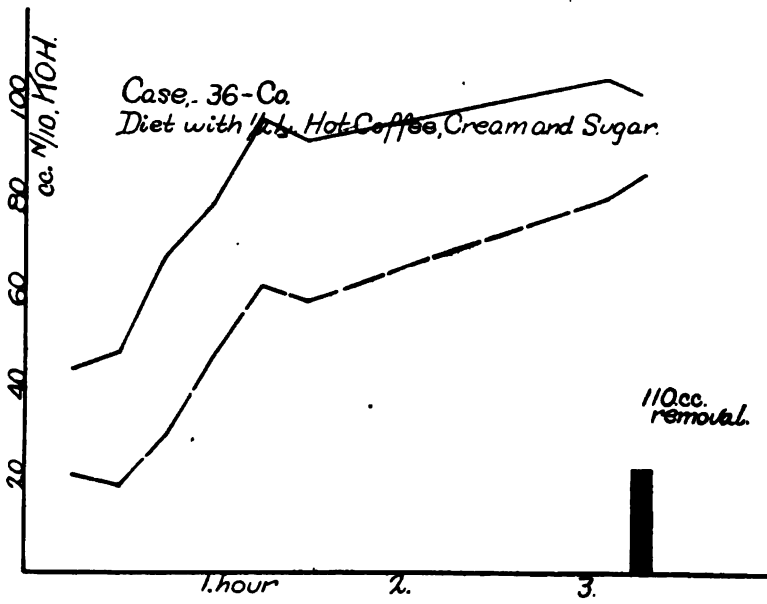


FIG. 5

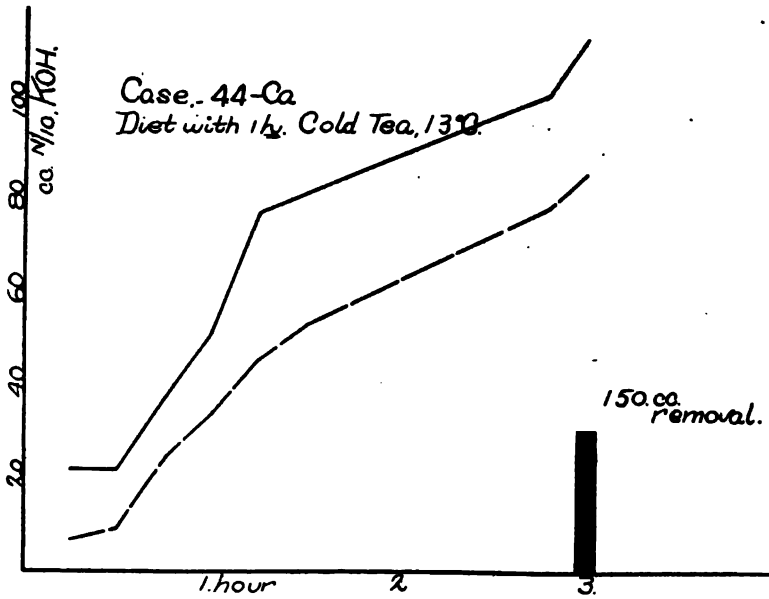


FIG. 6

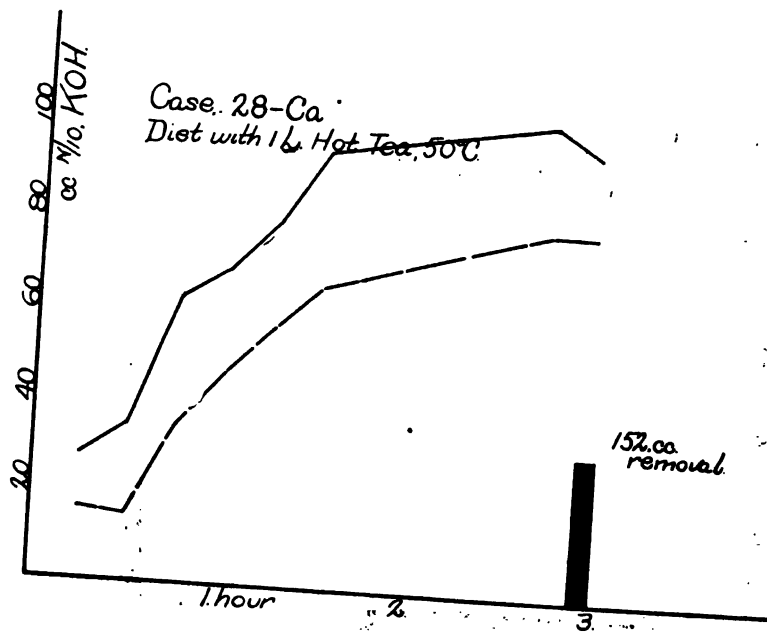


FIG. 7

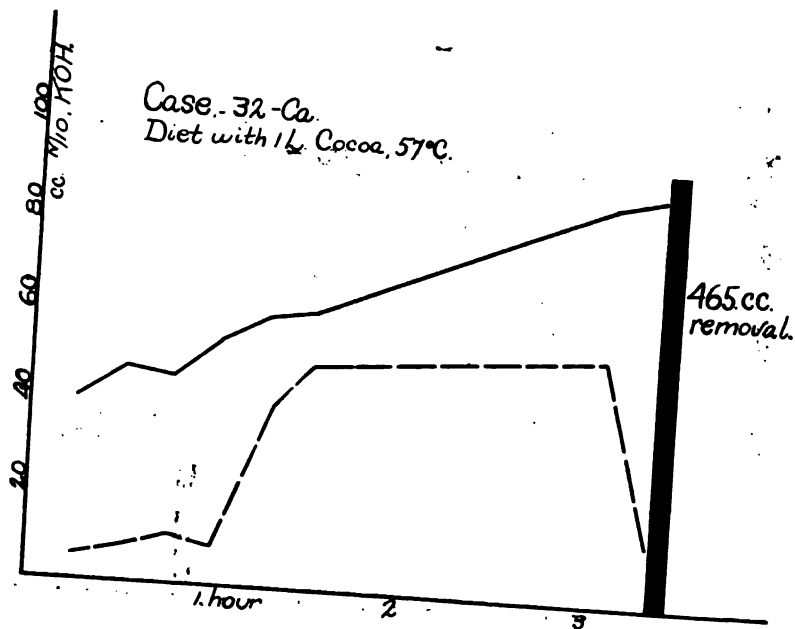


FIG. 8.

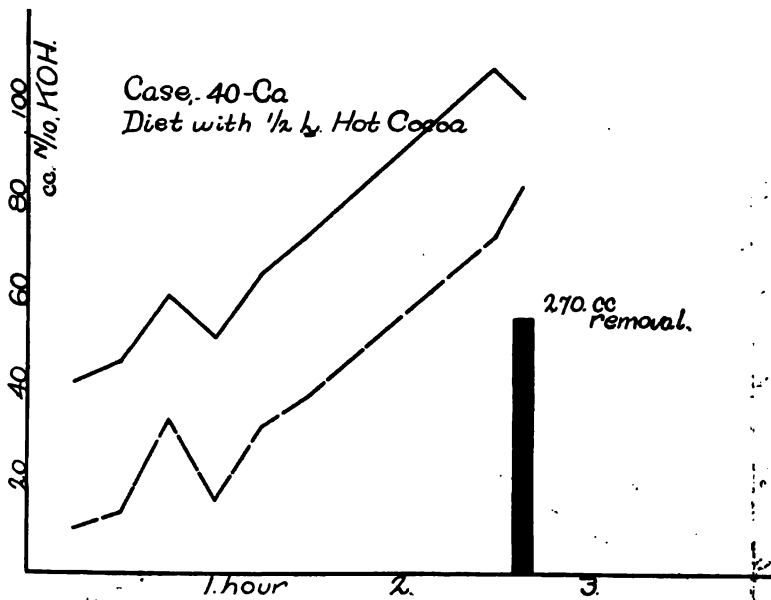


FIG. 9

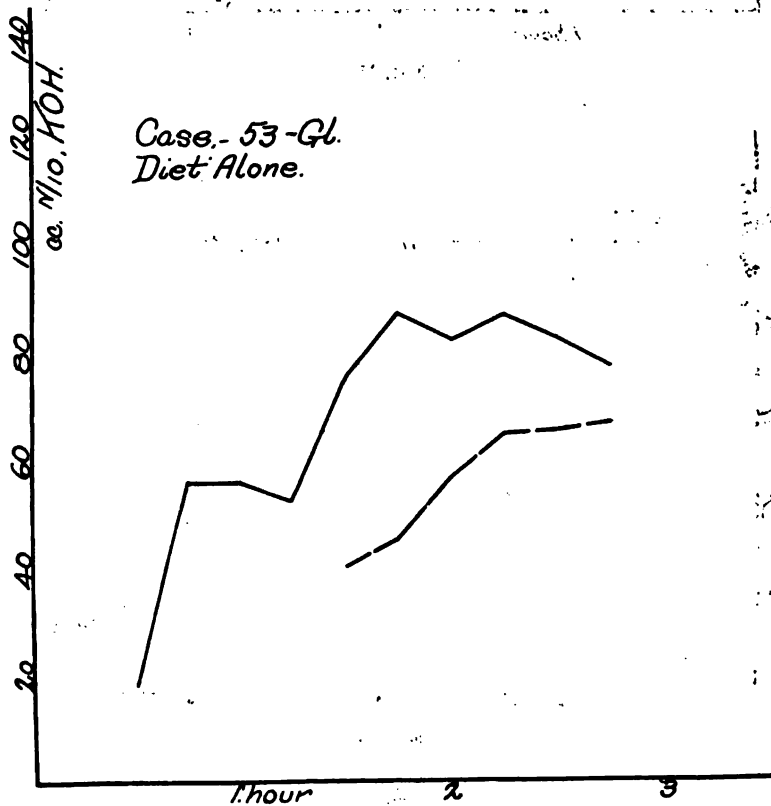


FIG. 10

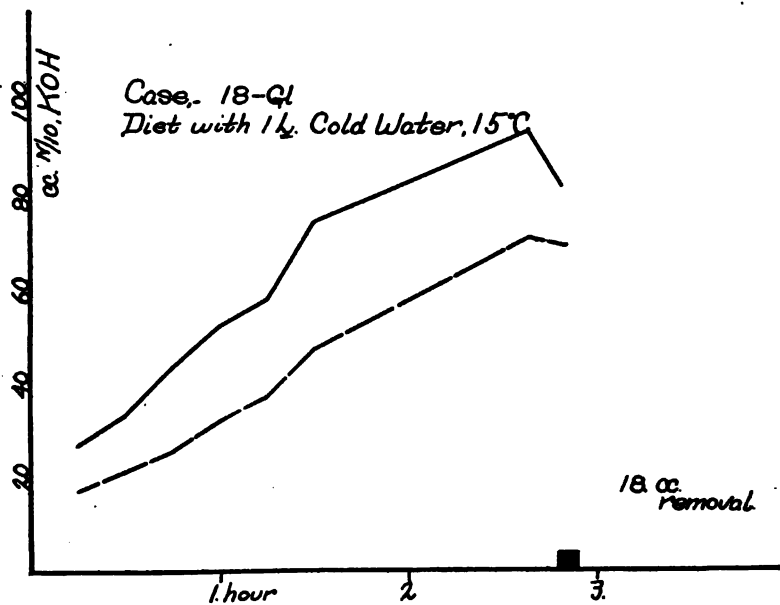


FIG. 11

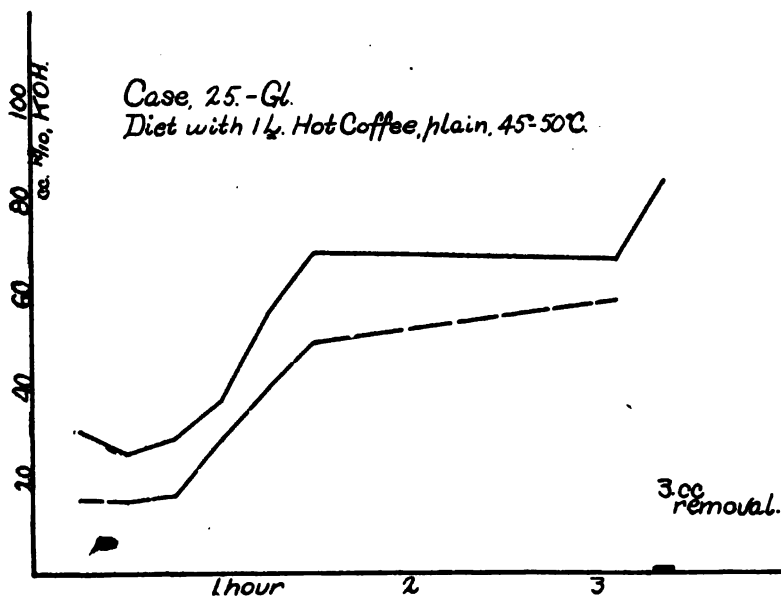


FIG. 12



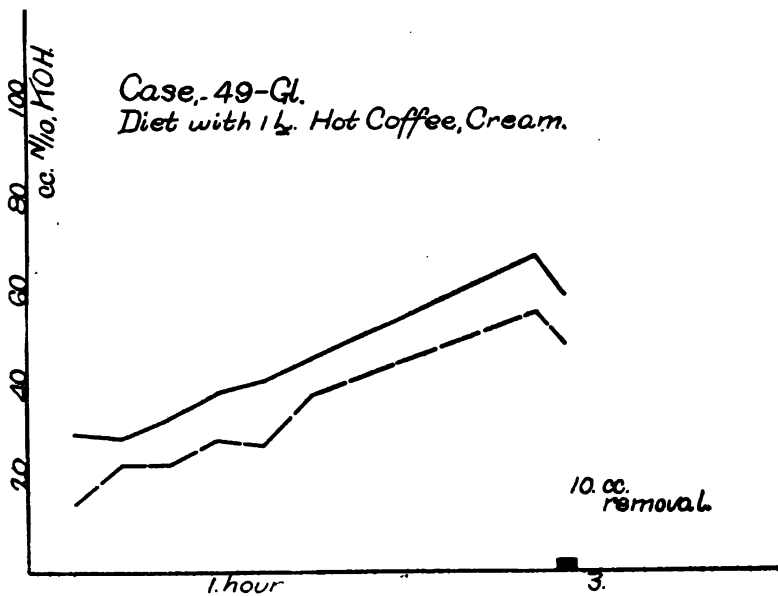


FIG. 13

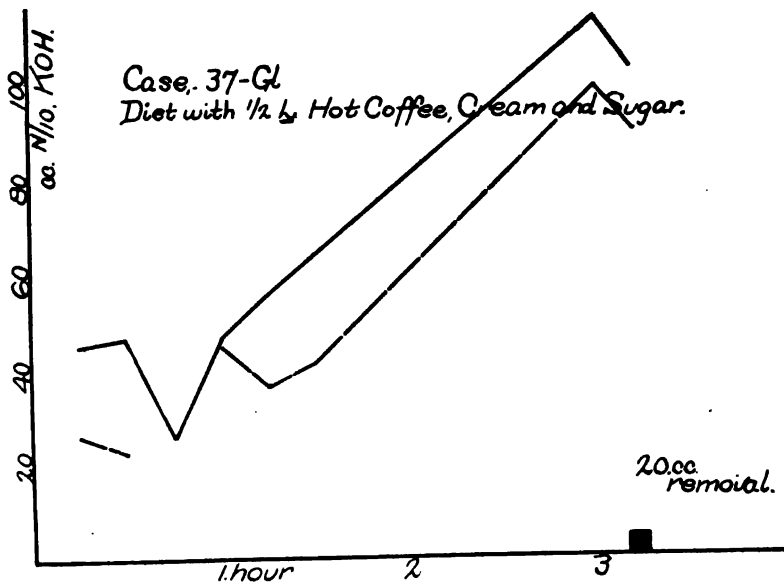


FIG. 14

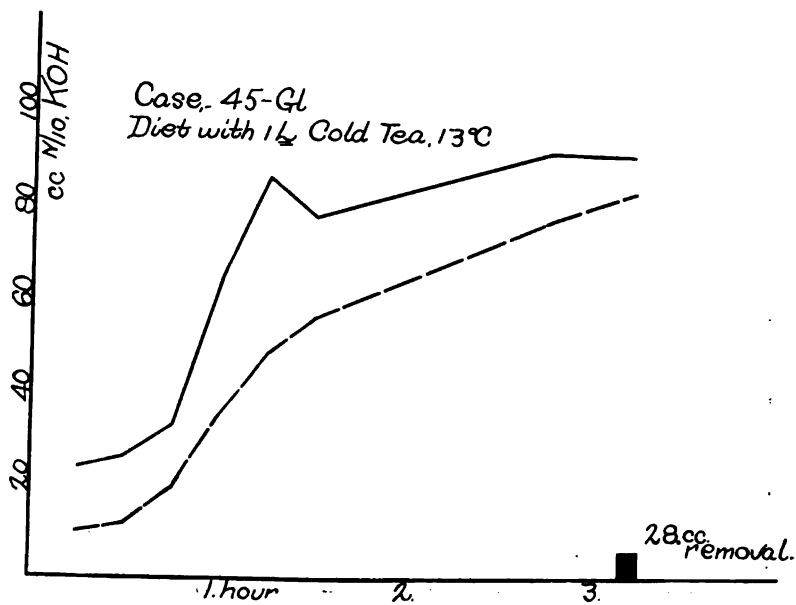


FIG. 15

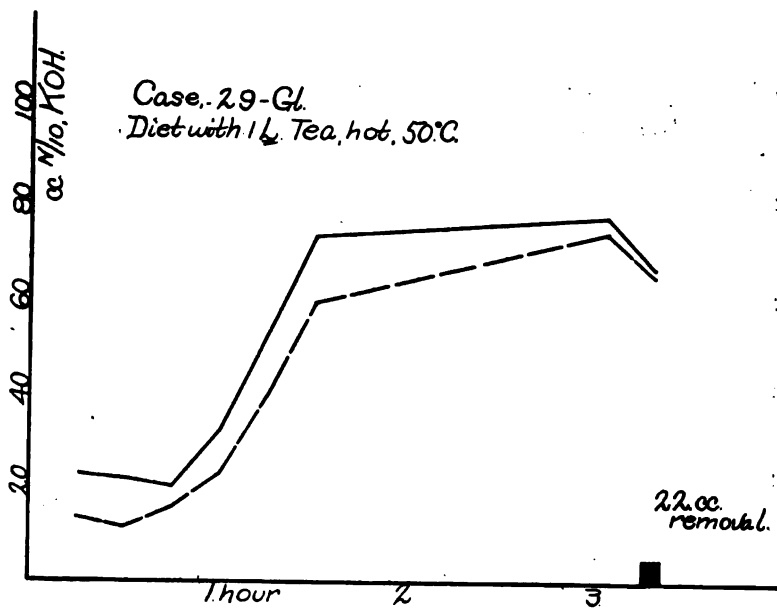


FIG. 16

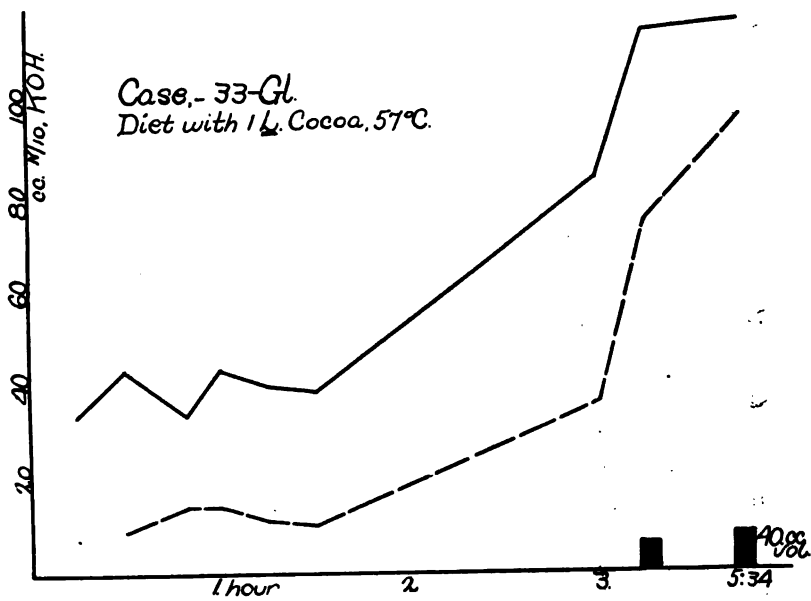


FIG. 17

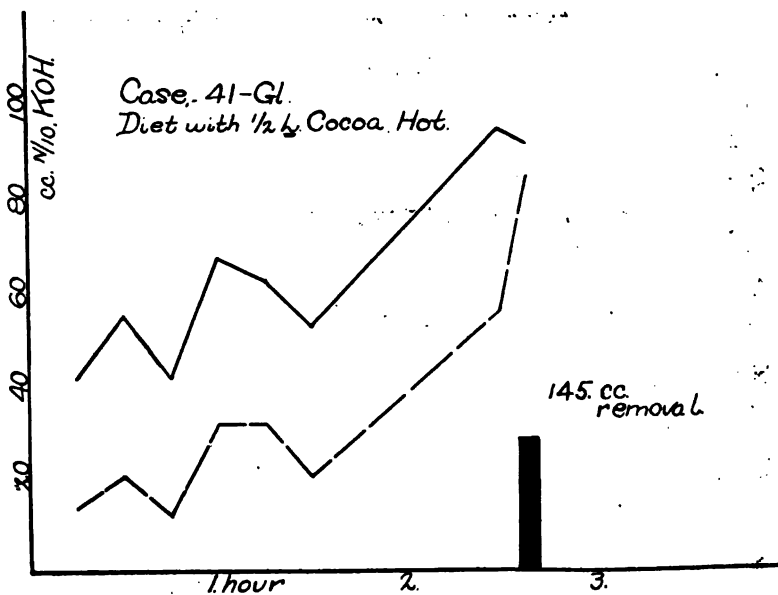


FIG. 18

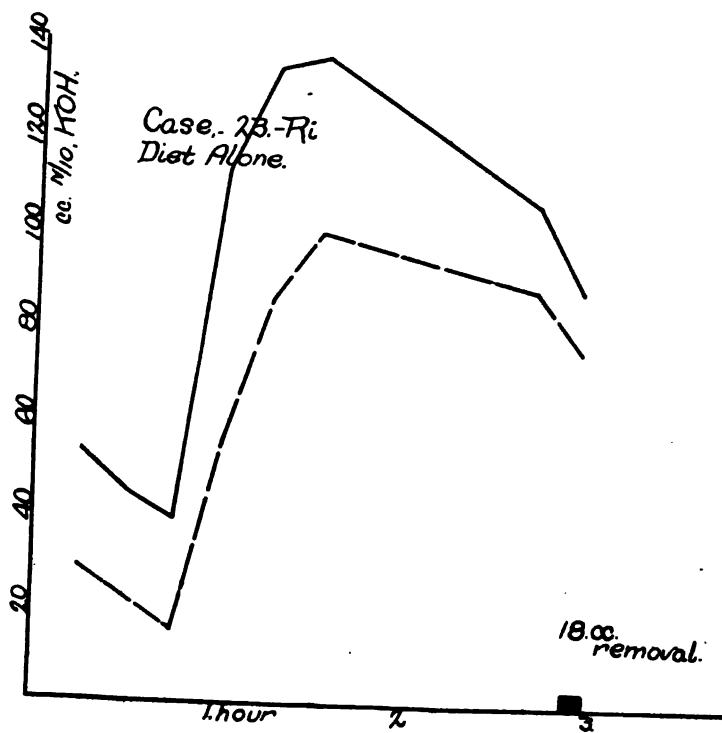


FIG. 19

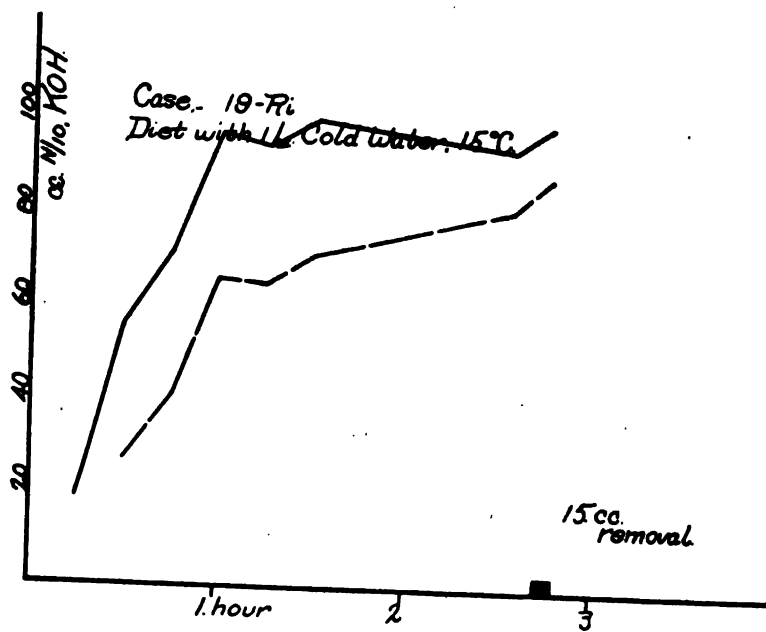


FIG. 20

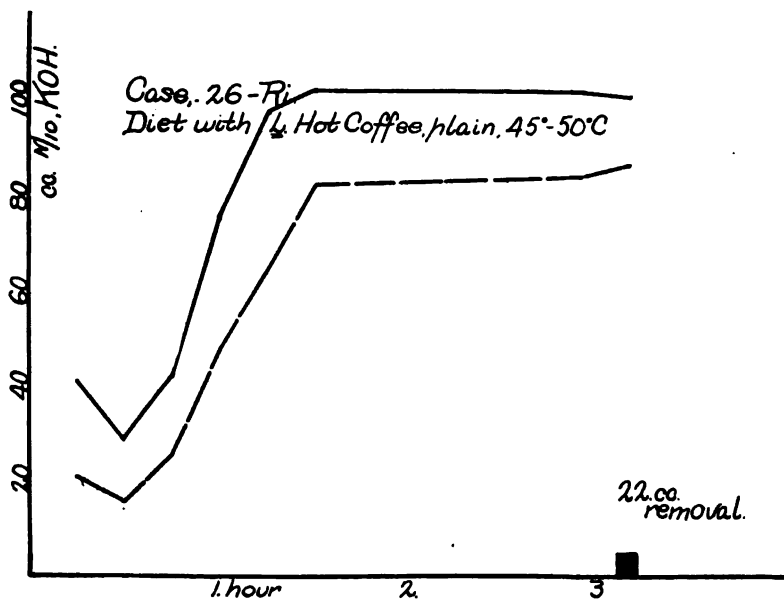


FIG. 21

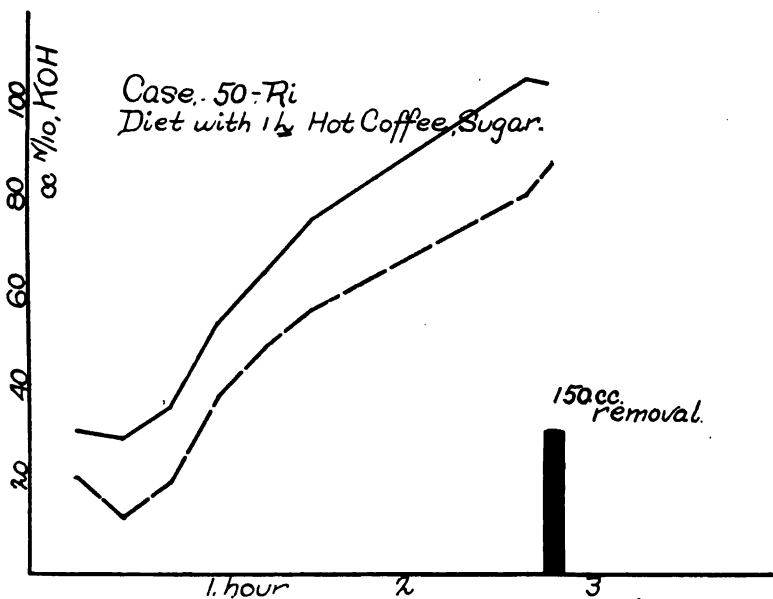


FIG. 22

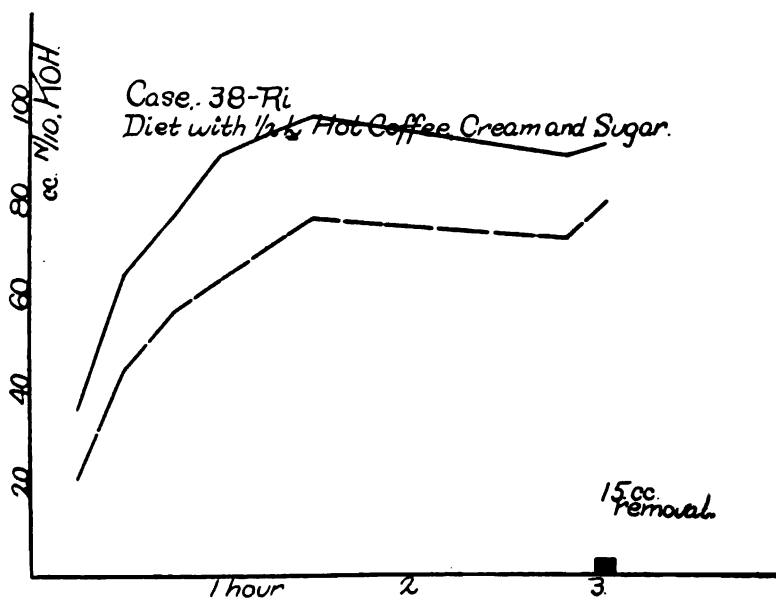


FIG. 23

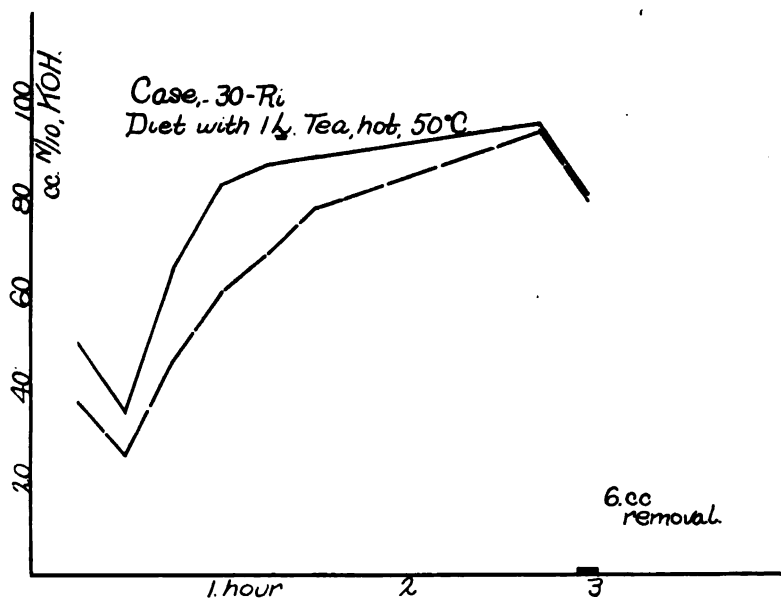


FIG. 24

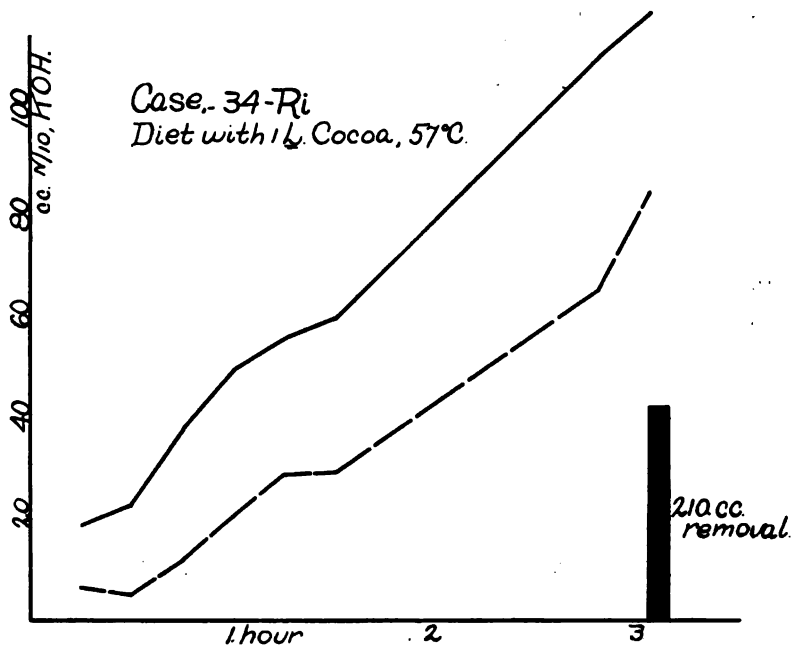


FIG. 25

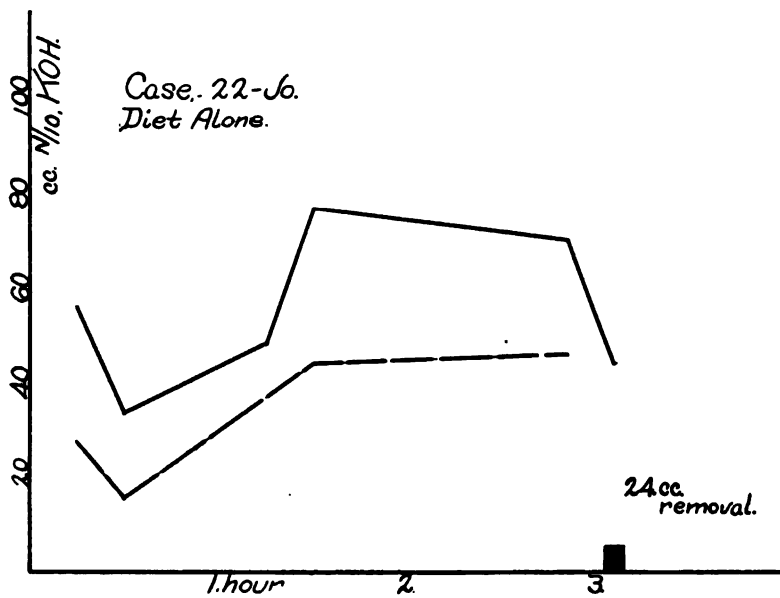


FIG. 26

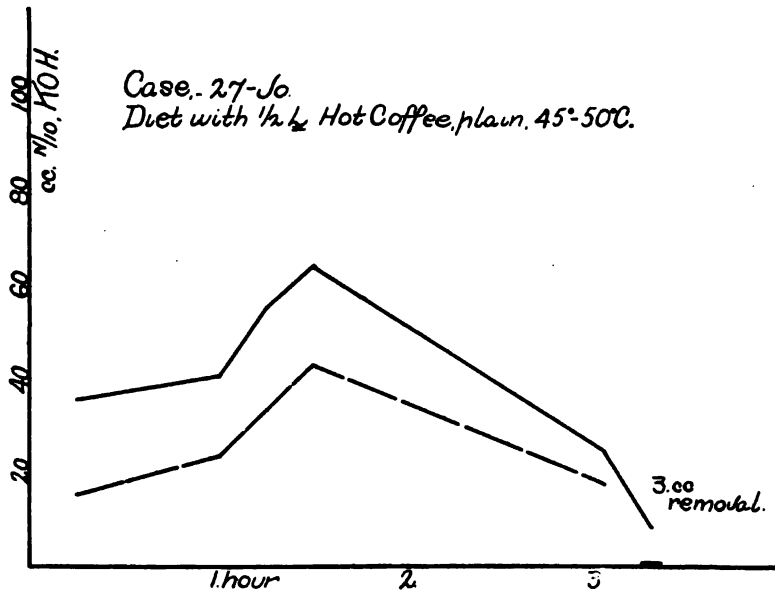


FIG. 27

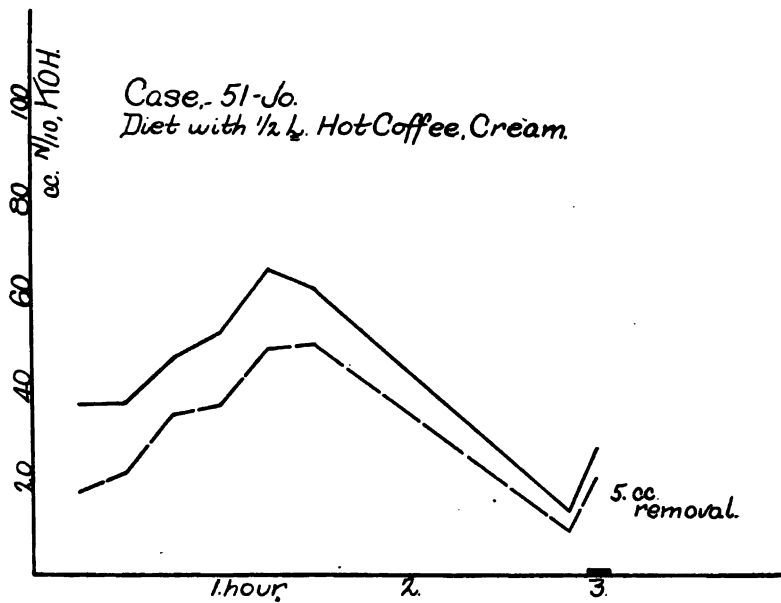


FIG. 28



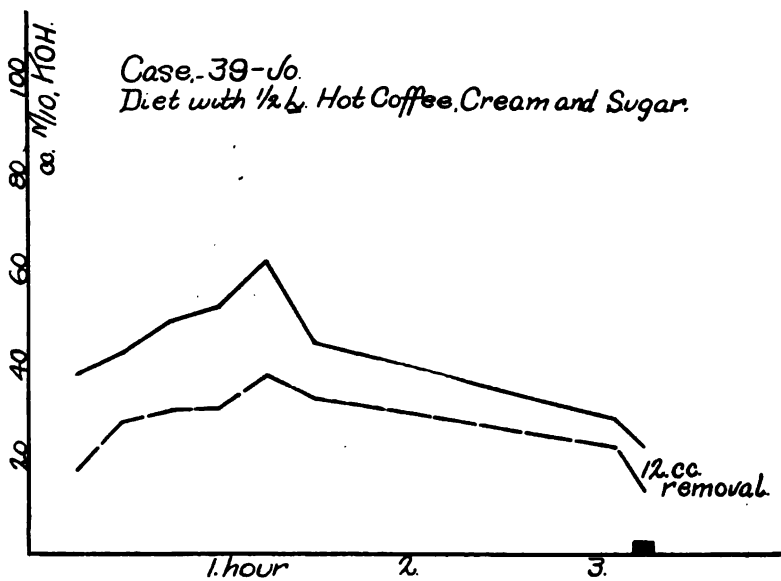


FIG. 29

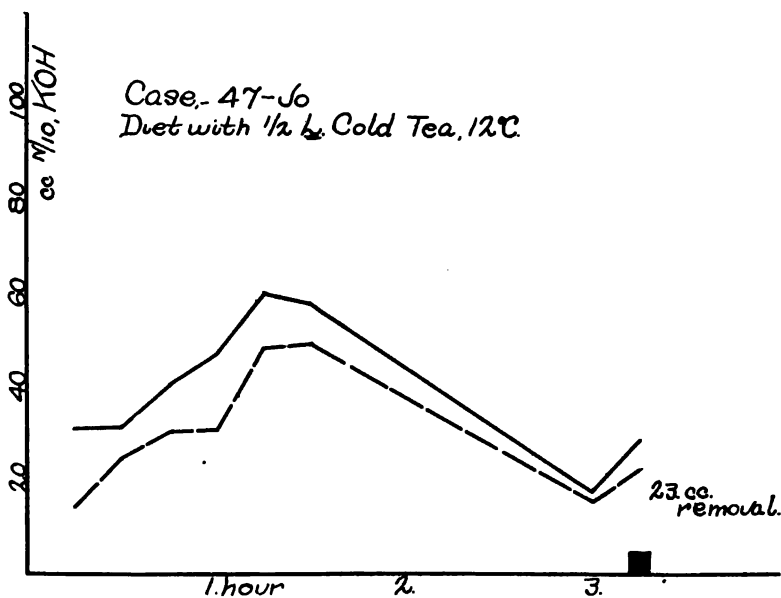


FIG. 30

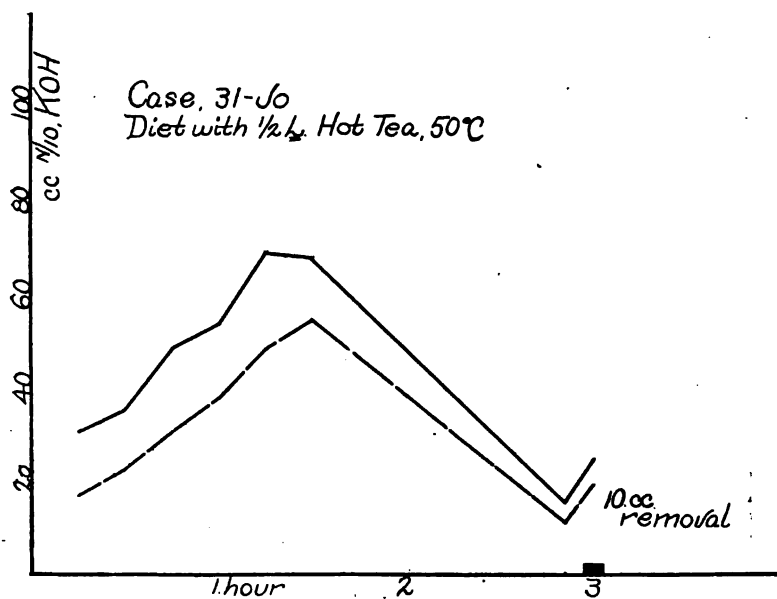


FIG. 31

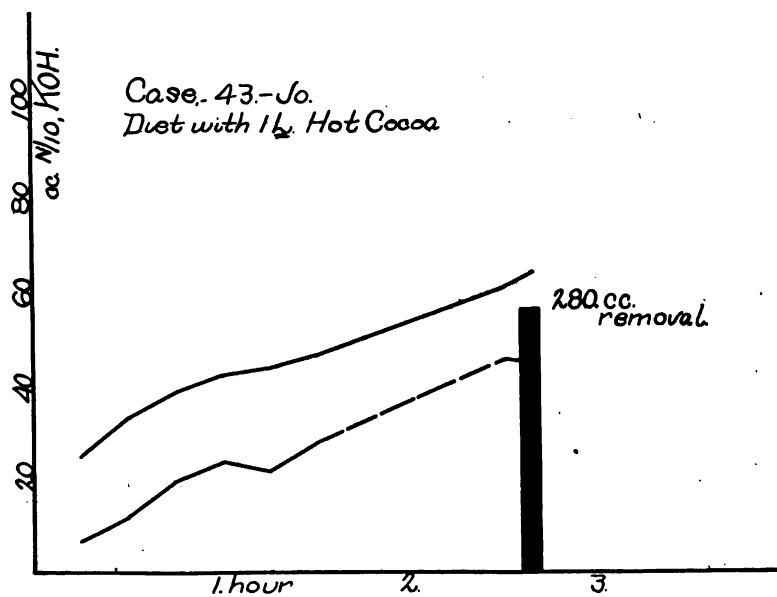


FIG. 32

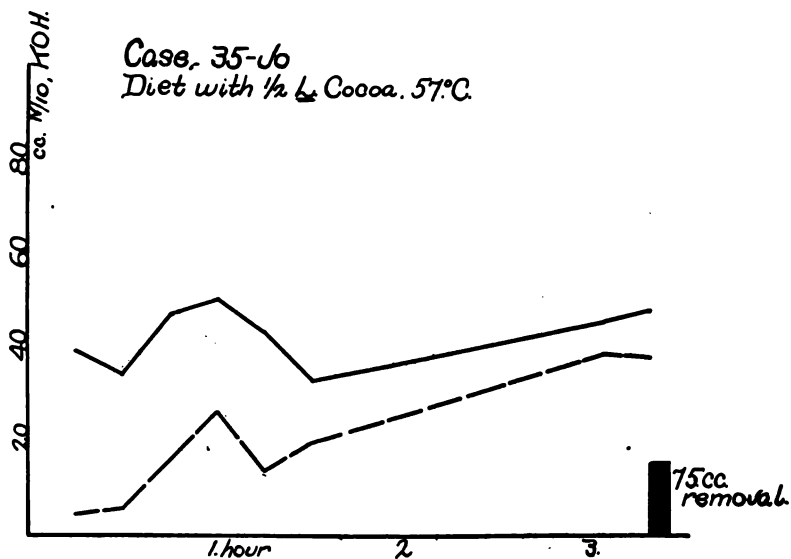


FIG. 33

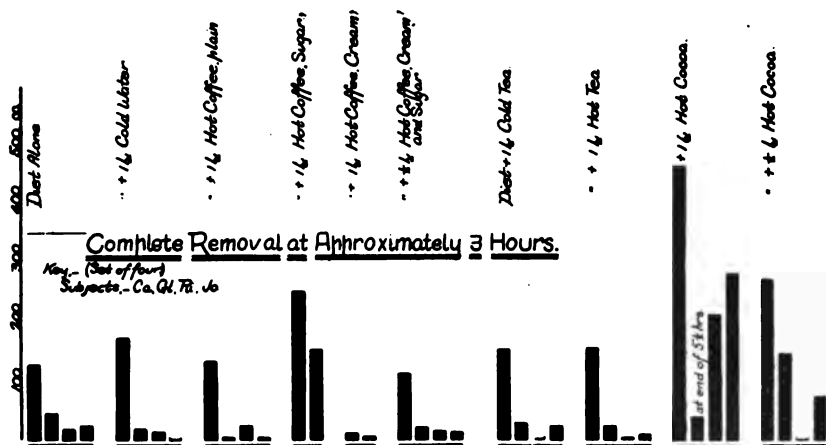


FIG. 34

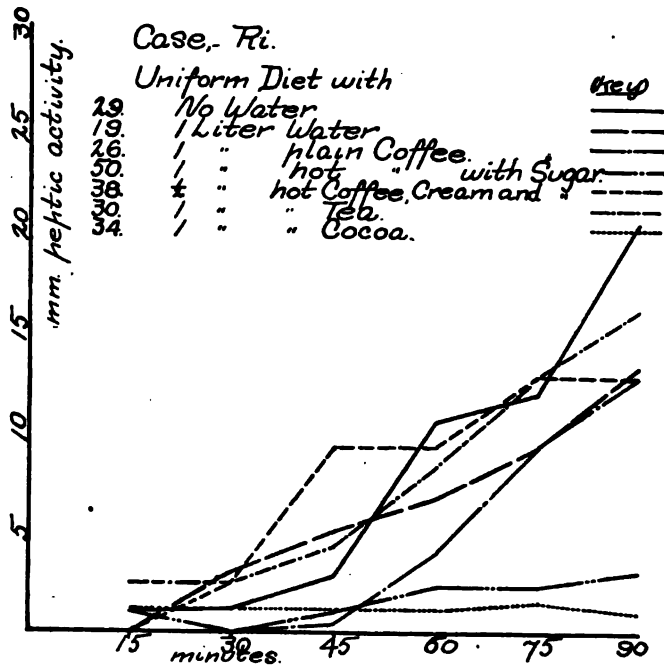


FIG. 35

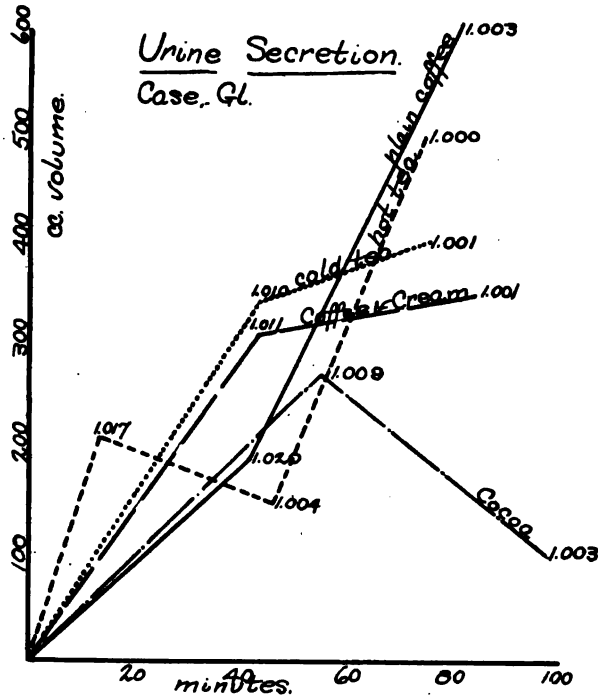


FIG. 36

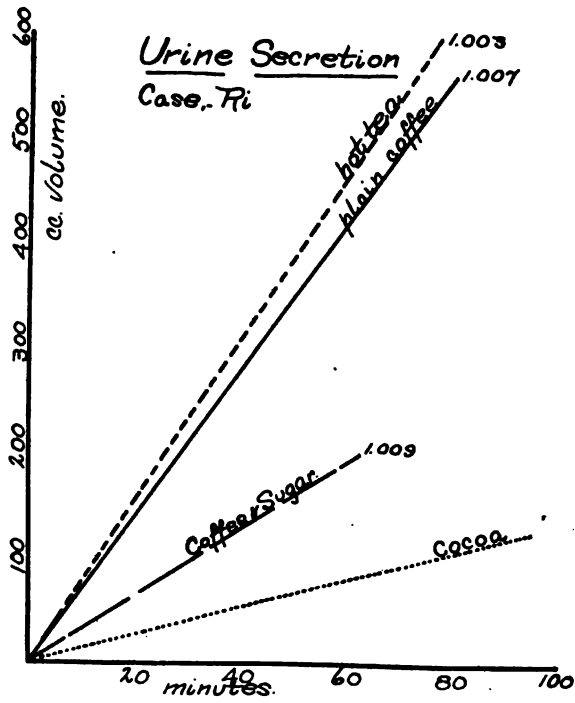


FIG. 37

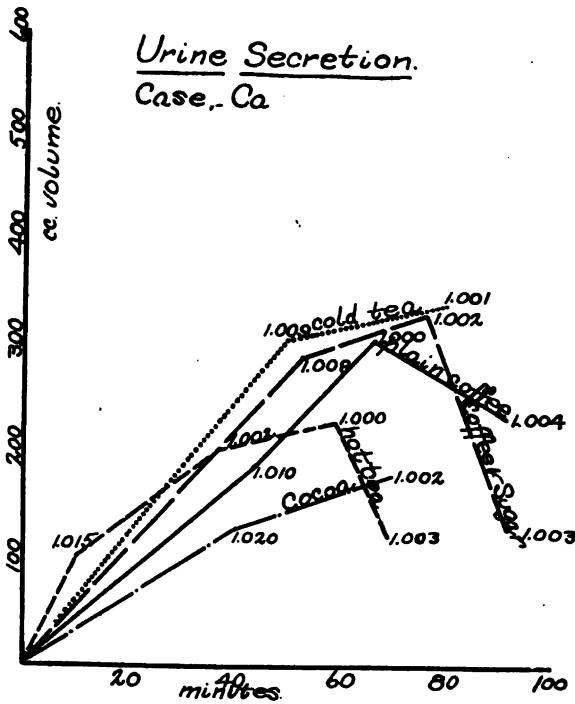


FIG. 38

## I. RAPID BLOOD PLASMA PROTEIN DEPLETION AND THE CURVE OF REGENERATION

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The published work of Kerr, Hurwitz and Whipple (1) brings out several facts about the blood serum proteins which may be mentioned briefly before we go on to a consideration of the experiments given below. The *stability of the serum protein concentration* is truly remarkable and obviously of some importance to the body physiology. The normal level is quite constant and considerable deviations from this normal base line are not well tolerated by the body; in fact, profound shock may result. When the serum proteins are depleted or washed out by the technique employed the repair or regeneration of these proteins is a slow process requiring from 5 to 10 days, depending upon the amount removed and other factors. It is as difficult to reconstruct these proteins as it is for the body to repair and replace liver cells following an extensive liver injury. It appears that the liver is especially concerned in the normal regeneration of blood serum proteins. Fasting does not prevent serum protein regeneration, therefore it is possible for the body to release these substances or to construct serum proteins from its own protein end products. There is no evidence that increased nitrogen breakdown is responsible for this regeneration of serum protein.

The experiments of Kerr, Hurwitz and Whipple were different as to method of blood serum depletion when compared with the experiments given below. These earlier experiments were mostly done by *interval bleedings* followed in each instance by a return of the washed red corpuscles suspended in modified Locke's solution. Under such circumstances a dog was bled 100 to 200 cc. and after washing by centrifugalization the same red cells were returned intravenously in Locke's solution. This procedure was repeated several times during the day until the serum protein depletion was carried to a minimum figure.

This method has been used by Abel, Rowntree and Turner (2) and called "plasmapheresis." It is obvious that this experimental procedure introduced wide fluctuations in blood volume and it was suspected that the shock which resulted was to be explained by the repeated hemorrhages and infusions.

We shall use the term *plasma depletion* or *plasmapheresis* to indicate a removal of plasma proteins by means of repeated hemorrhage followed or accompanied by the replacement of like amounts of red cells suspended in a protein-free fluid. The plasma depletion in our experiments was effected by a method first introduced by Morawitz (3); bleeding and the removal of whole blood was simultaneous with the replacement of the red cell Locke's solution mixture. The inflow and outflow volume was at all times constant and obviated any fluctuation in blood volume. All aseptic precautions were taken in manipulation, washing and final preparation of the red cell mixtures. The red cell mixtures were introduced at body temperature and the dog was kept warm during the experiment.

The method employed in these experiments enables an investigator to reduce the blood plasma proteins from the normal level of 5 to 6 per cent to a very low level of 1.5 to 0.9 per cent. This can be done in a matter of minutes (2 to 10 minutes) leaving the animal uninfluenced by the large and numerous fluctuations in blood volume and oxygen-carrying capacity of the blood which undoubtedly occur in the method used by Kerr, Hurwitz and Whipple. In addition it facilitates observations of that portion of the curve of protein regeneration immediately following a large single depletion and permits observations on an uninterrupted regeneration curve.

#### METHOD

The animals used were sound young dogs maintained on a mixed diet. In most cases no food was given the animal for a period of 12 hours preceding the experiment. Free access to water obtained. Under complete ether anesthesia and with aseptic precautions an incision was made either into the region of the femoral vessels or of the large vessels of the neck. The artery and vein were exposed and clamped. Into each was introduced a vaseline-coated cannula pointing toward the heart. Plasma removal was effected by withdrawing through the cannula placed in the artery large quantities of blood. This blood was allowed to flow into a graduated bottle. Simultaneously, a suspension

of washed corpuscles warmed to body temperature was injected under pressure through the venous cannula. This suspension was delivered from a flask which was also graduated. The graduations were used to permit a comparison of the inflow with outflow to be made at any time during the exchange. In this way inflow and outflow were observed and kept equal at all times. In order to maintain an even suspension of the injection mass, the latter was frequently shaken.

The fluid injected consisted of washed dog corpuscles suspended in a modified Locke's solution in the ratio of three parts packed corpuscles to two parts by volume of the saline mixture. The composition of the Locke's solution was: sodium chloride, 0.9 per cent; potassium chloride, 0.042 per cent; sodium bicarbonate, 0.02 per cent. The corpuscles in all cases were obtained from the blood of healthy dogs. This blood was drawn into sodium oxalate, centrifugalized, and the plasma removed from the sedimented corpuscles. The corpuscles were then washed twice in the modified Locke's solution by resuspension and centrifugalization. Aseptic precautions were observed in all these manipulations.

The exchange was effected in a period ranging in different animals from 2 to 25 minutes. The amount of fluid withdrawn varied in individual experiments from 60 per cent to 195 per cent of the animal's blood volume. The amount of blood simultaneously injected corresponded within a few cubic centimeters to the amount withdrawn. The blood volume was estimated as 10 cc. per 100 grams of body weight. In a number of experiments the actual blood volume was kindly determined for us by Dr. C. W. Hooper, using a dye method recently described. This paper (4) shows that the blood volume of active normal dogs as determined by the dye method is approximately 10 cc. per 100 grams of body weight.

At the end of the operative procedure the cannulae were withdrawn and the vessels ligated. Vaseline was applied liberally to the wound. In a few instances it was necessary to make a single suture through the subcutaneous tissues at the site of operation. In practically every experiment the wound healed quickly with little or no suppuration.

Samples of blood were collected through the arterial cannula at the beginning and at the end of the exchange and again 15 minutes later. Subsequent samples were taken from the jugular vein by means of a needle and syringe. On each of these occasions two samples were withdrawn. One of these was drawn into a 15 cc. hematocrit tube containing 3 cc. of 1 per cent sodium oxalate. The other sample was drawn into a plain heavy-walled glass test tube and allowed to clot. Both



of these samples were then centrifugalized at a high rate of speed. The serum from the clotted sample was then used for estimation of serum albumins, serum globulins and the non-protein fraction by the refractometric method of Robertson (5). Percentage corpuscles readings were made from the oxalated sample, correction being made for the amount of oxalate solution present. The plasma from this sample was also used for the determination of fibrin. This was carried out according to the method of Cullen and Van Slyke (6). This method consists in diluting 5 cc. of plasma in 100 cc. of salt solution. To this mixture 1.5 cc. of a 2.4 per cent solution of  $\text{CaCl}_2$  was added to supply calcium and to promote clotting, and a Kjeldahl done on the mass of fibrin obtained.

The clinical condition of the animal was closely observed. The rectal temperature, rate of respiration, pulse, diarrhea and vomitus, as well as the general appearance of the animal, were noted. In cases in which death resulted, careful autopsies were performed at once. The clinical condition of the animal will be made the subject of a subsequent paper with a discussion of the peculiar type of shock which may develop under these conditions.

#### EXPERIMENTAL OBSERVATIONS

This paper in general deals with the recovery experiments but in certain tables we include many of the lethal shock experiments (tables 12, 13 and 14). For the sake of comparison we give in table 1 a type experiment which was followed promptly by fatal shock. Many of these experiments will be found in the next paper of this series and in that place the general discussion of this peculiar shock will be presented. It will be noted in table 1 that the reduction of total proteins, albumin and globulin is pretty uniform and is a fall to approximately one-third of normal. The emergency increase of protein during the 15 minutes following the plasma depletion is not as marked as usual (see table 2). There is no further increment of serum protein in the hour following this 15-minute sample and this may be explained in part by the profound shock. The content of red cells in whole blood as shown by the hematocrit is lower than usual and the fall which appears immediately after plasmapheresis would indicate the use of a red cell mixture containing fewer red cells than intended. This factor does not complicate the remaining experiments and we believe has no significance.

*Experiment 104.* (See table 1). 122 per cent exchange.

*Dog 18-48.* Young female bull dog. Weight 16.9 pounds. Blood volume on November 20, 1917 (by dye method) was 941 cc.

*November 20, 1917.* Under ether anesthesia 940 cc. blood were withdrawn from the left carotid artery. Simultaneously 1000 cc. of blood corpuscle suspension were injected into the left external jugular vein. The duration of the exchange was 10.5 minutes. Animal showed almost immediately a great fall in pulse pressure and arterial tension. Profound depression with forced irregular respiration developed in about an hour. Death 2 hours after the exchange. The autopsy findings are uniform in all fatal experiments and will be described in detail in the following paper.

TABLE 1

*122 per cent blood volume exchange; dog 18-48; experiment 104*

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	HEMATOCRIT RED CELL PER CENT	REMARKS
	Total protein	Albumin	Globulin	Non-protein			
Before exchange.....	5.7	4.2	1.5	2.0	0.25	47	
Immediately after....	1.9	1.4	0.5	1.6	0.12	35	Considerable hemolysis
15 minutes after.....	2.3	1.9	0.4	1.7	0.11	38	Considerable hemolysis
1 hour and 20 minutes.....	2.4	2.0	0.4	2.1		30	Fatal shock

Table 2 gives the results of an experiment which contrasts with this lethal shock experiment (table 1). The second experiment presents an even large blood exchange in plasmapheresis but this dog is not disturbed by the procedure. It will be noted that there are marked individual differences in dogs as to their tolerance to this plasma depletion. Any given dog will show a considerable uniformity of reaction to a unit exchange but must be standardized to ascertain this reaction. Table 2 also shows a fall of total protein, albumin and globulin to about one-third normal following the plasmapheresis. We wish to call attention to the emergency increase in blood proteins which appears *within 15 minutes*. This is a characteristic reaction which obtains in practically all experiments (tables 11 and 12). The increase in serum proteins during the next 24 hours is very marked and exceeds 1 per cent protein—equivalent to more than 20 per cent of the total protein replaced in the blood serum. Subsequent regeneration of protein in the serum is slow and requires several days for complete recovery.

*Experiment 69.* (See table 2). 170 per cent exchange.

*Dog 18-9.* Young female bull dog. Weight 14 pounds. Blood volume on July 19, 1917 (by dye method) was 772 cc.

*August 2, 1917.* Under ether anesthesia 1081 cc. blood were withdrawn from the right femoral artery. Simultaneously 1081 cc. of blood corpuscle suspension were injected into the right femoral vein. The duration of exchange was 12 minutes. Following the exchange the temperature fell about 2 degrees, but returned to the original level within 2 hours. Pulse and respiration were fair at all times.

A much smaller volume exchange is shown in table 3, yet considerable shock resulted. It will be seen that the level of total proteins, albumin and globulin falls to approximately one-half of normal corresponding to the smaller exchange volume (90 per cent). There is a

TABLE 2

*170 per cent blood volume exchange; dog 18-9; experiment 69*

TIME	BLOOD SERUM READINGS IN PER CENT				REMARKS
	Total protein	Albumin	Globulin	Non-protein	
Before exchange.....	5.6	3.8	1.8	1.7	
Immediately after.....	2.0	1.3	0.7	1.4	Moderate hemolysis
15 minutes after.....	2.9	2.0	0.9	1.3	Moderate hemolysis. No shock
2d day.....	4.2	3.1	1.1	1.9	Slight hemolysis. Normal
3d day.....	4.5	2.5	2.0	2.1	
4th day.....	4.8	3.2	1.6	1.7	

moderate increase in serum proteins during the 15-minute period and less than usual during the first 24 hours following plasmapheresis. We cannot explain satisfactorily the remarkable drop in red cell hematocrit which is present after 11 hours and persists many days. Possibly the red cells used for infusion in this experiment had been seriously injured and went to pieces in the circulation. In confirmation of this suggestion we note the presence of hemolysis in blood samples taken on the first four days following the experiment. It may be suspected that an hemolysin was present in this dog's blood but there is reasonable doubt whether hemolysins actually do occur in the dog in sufficient amount to destroy large numbers of homologous red cells.

*Experiment 103.* (See table 3). 91 per cent exchange.

*Dog 18-66.* Young female bull mongrel. Weight 17 pounds.

November 15, 1919. Under ether anesthesia 700 cc. blood were withdrawn from the right femoral artery. Simultaneously 700 cc. of blood corpuscle suspension were injected into the right femoral vein. The duration of exchange was 9 minutes. Following the exchange the temperature showed little or no alteration from the original level. The pulse was regular but poor in tension for a number of hours. One-half cubic centimeter of adrenalin subcutaneously was given 4 hours after the operation. After 24 hours the animal was in excellent condition.

In all these experiments the washing out of plasma proteins is accomplished by a *rapid exchange*. The bleeding and simultaneous infusion of the red cell mixture occupies only a few minutes, the limits being

TABLE 3  
91 per cent blood volume exchange; dog 18-86; experiment 103

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	HEMATOCRIT RED CELL PER CENT	REMARKS
	Total protein	Albumin	Globulin	Non-protein			
Before exchange.....	6.2	4.5	1.7	2.0	0.42	49	
Immediately after....	3.2	2.2	1.0	1.5		49	
15 minutes after.....	3.8	2.9	0.9	1.6	0.21	56	
3 hours.....	4.3	3.0	1.3	1.7	0.58	50	Moderate shock
11 hours.....	4.0	2.9	1.1	2.3	0.47	33	
2d day.....	4.3	2.8	1.5	2.6	0.48	35	Dog normal.
3d day.....	4.8	3.7	1.1	2.5	0.47	29	Hemolysis
5th day.....	4.5	3.1	1.4	3.0	0.41	27	Hemolysis
6th day.....	5.3	3.9	1.4	2.3			
8th day.....	5.4	3.8	1.6	2.9	0.45	32	
10th day.....	5.7	4.1	1.6	2.0	0.42	32	
12th day.....	5.4	3.9	1.5	2.1	0.56	32	

2 to 25 minutes. Within these limits the speed of exchange, whether 2 minutes or 25 minutes, seems to make little difference. To make this point clear we may contrast tables 4 and 5. The first of these two experiments done on the same animal (table 4) shows the reaction following an exchange of 100 per cent done in 14 minutes. There was definite shock but a rapid recovery. The second experiment done on this dog after an interval of 2 weeks to insure complete recovery, shows the reaction following a very rapid 100 per cent exchange which was completed *within 2 minutes*. There was if anything less shock on this occasion than after the first exchange. It is interesting to note how closely

the curves of total protein, albumin and globulin in the two experiments coincide. The prompt rise in the 15-minute interval is identical and the initial fall corresponds to the other experiments discussed.

*Experiment 93.* (See table 4). 99 per cent exchange.

*Dog 18-48.* Young female bull dog. Weight 13.3 pounds.

*October 4, 1917.* Under ether anesthesia 600 cc. blood were withdrawn from the right femoral artery. Simultaneously 700 cc. of blood corpuscle suspension

TABLE 4

99 per cent blood volume exchange; dog 18-48; experiment 93

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	HEMATOCRIT RED CELL PER CENT	REMARKS
	Total protein	Albumin	Globulin	Non-protein			
Before exchange.....	4.5	3.2	1.3	2.2	0.26	58	Definite shock
Immediately after....	2.0	1.3	0.7	1.6	0.16	58	
15 minutes after.....	3.3	2.6	0.7	1.5	0.15	58	
9½ hours.....	3.9	3.0	0.9	1.8	0.22	46	Normal
2d day.....					0.18	54	
3d day.....	4.0	3.1	0.9	1.9	0.30	42	
4th day.....	4.4	3.3	1.1	1.7	0.39		

TABLE 5

104 per cent blood volume exchange; dog 18-48; experiment 96

TIME	BLOOD SERUM READINGS IN PER CENT				REMARKS
	Total protein	Albumin	Globulin	Non-protein	
Before exchange.....	6.4	3.5	2.9	1.7	Slight shock
Immediately after....	2.1	1.3	0.8	1.3	
15 minutes after.....	3.2	2.2	1.0	1.6	
10 hours.....	3.9	2.7	1.2	1.6	Normal
2d day.....	4.5	2.6	1.9	1.9	

were injected into the right femoral vein. The duration of exchange was 14 minutes. Animal showed definite signs of intoxication after about an hour following the exchange, with some bloody feces after about 5½ hours. After 24 hours animal appeared to have recovered completely.

*Experiment 96.* (See table 5). 104 per cent exchange.

*Dog 18-48.* Young female bull dog. Weight 13.8 pounds. "Plasmapheresis," 99 per cent exchange with a duration of 14 minutes, done on October 4 (exper. 93, table 4). Showed definite signs of intoxication.

October 18, 1917. Under ether anesthesia 650 cc. blood were withdrawn from the left femoral artery. Simultaneously 650 cc. of blood corpuscle suspension were injected into the left femoral vein. The duration of exchange was 2 minutes. Immediately following the exchange there was a slight transient fall in temperature. There was no immediate alteration of the pulse; however after about an hour the pulse was of poor volume and the animal appeared decidedly dull. Bloody feces were noted at this time. After 24 hours the animal appeared to be in good condition.

Table 6 gives valuable data concerning the speed of exchange in its relation to shock and the curve of protein regeneration. In this experiment the blood volume exchange of 75 per cent was completed in 2.5 minutes. There was no shock and we may compare the previous exchanges done on this same animal (Sept. 12, 1917, exper. 84, 80 per

TABLE 6  
75 per cent blood volume exchange; dog 18-35; experiment 94

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	HEMATOCRIT RED CELL PER CENT	REMARKS
	Total protein	Albumin	Globulin	Non-protein			
Before exchange.....	5.4	3.8	1.6	1.9	0.21	40	
Immediately after.....	3.0	2.2	0.8	1.5	0.19	48	
15 minutes after.....	3.7	2.7	1.0	1.8	0.23	54	
3½ hours.....	4.6	3.4	1.2	1.8	0.26	61	No shock
6½ hours.....	4.7	3.5	1.2	1.8	0.30	44	
9½ hours.....	4.1	2.9	1.2	1.5			Normal
2d day.....	4.9	3.6	1.3	1.9	0.44		
3d day.....	4.4	3.1	1.3	2.8			
4th day.....	4.6	2.4	2.2	2.4			

cent exchange, shock very slight, time 7 minutes; October 3, 1917, exper. 92, 74 per cent exchange, no shock, time 14 minutes). The curve of serum protein depletion and regeneration is similar to other experiments. The small per cent exchange lowers the total protein to 3.0 per cent and the emergency increase is definite within 15 minutes, giving a rise to 3.7 per cent. It appears that the emergency reaction by which a considerable amount of serum protein is thrown into the blood stream, can be called out by a large or small exchange using this method.

Experiment 94. (See table 6). 75 per cent exchange.

Dog 18-35. Young female bull dog. Weight 16 pounds. "Plasmapheresis," 80 per cent exchange, done in 7 minutes on September 12, 1917, showing very

slight shock (exper. 84); another 14 per cent exchange done in 14 minutes on October 3, 1917, showing no shock (exper. 92). Blood volume on October 9, 1917 (by dye method) was 671 cc.

*October 11, 1917.* Under ether anesthesia 545 cc. blood were withdrawn from the right carotid artery. Simultaneously 545 cc. of blood corpuscle suspension were injected into the right external jugular vein. The duration of the exchange was 2½ minutes. Animal showed a transient drop in temperature of 3 degrees immediately following the exchange. Animal showed little or no signs of intoxication.

Table 7 shows a remarkably prompt return to normal after a large exchange (109 per cent). The total proteins fell to a level of 50 per cent normal which is a normal reaction. We cannot explain the figures, which appear to show a peculiar reaction on the part of the albumin and globulin fractions. These peculiar reactions will appear at

TABLE 7

*109 per cent blood volume exchange; dog 18-20; experiment 89*

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	HEMATOCRIT RED CELL PER CENT	REMARKS
	Total protein	Albumin	Globulin	Non-protein			
Before exchange.....	5.0	3.6	1.4	1.9	0.35	50	
Immediately after...	2.5	0.9	1.6	1.4	0.12	60	
15 minutes after.....	3.0	1.5	1.5	1.6	0.11	73	
4½ hours.....	4.2	2.2	2.0	1.7	0.13	73	Very slight shock
2d day.....	5.1	2.9	2.2	2.1		41	Normal

times in spite of every care used in the method, but we are inclined to suspect technical errors as in part responsible. The return of the total protein to normal within 24 hours is unusual and would indicate an unusually large emergency reserve. The peculiar rise in red cell hematocrit will be found in this experiment and in a few subsequent experiments. That it appears in the 15-minute and 4-hour samples but not in the sample taken immediately after the exchange is very perplexing. There is no severe shock to account for any withdrawal of fluid from the blood. We have no convincing explanation to offer.

*Experiment 89.* (See table 7). 109 per cent exchange.

*Dog 18-20.* Young female bull dog. Weight 16.1 pounds.

*September 20, 1917.* Under ether anesthesia 800 cc. blood were withdrawn from the femoral artery. Simultaneously 800 cc. of blood corpuscle suspension were injected into the femoral vein. The duration of the exchange was 14½

minutes. Animal showed little or no depression. The temperature fell 3 degrees, however, and the animal shivered considerably for 8 or 9 hours. In good condition after 24 hours.

Table 8 shows another typical experiment giving the usual curve of blood proteins following plasmapheresis of moderate amount (90 per cent). The *fibrin curve* is given in this experiment and we believe this illustrates the usual reaction on the part of this plasma globulin. The method used gives certain opportunities of error when small amounts of plasma are analyzed for fibrin. More work in this field has been completed by Mr. Foster in this laboratory and will soon be published. We do not wish to put too much emphasis on these figures.

TABLE 8  
90 per cent blood volume exchange; dog 18-68; experiment 105

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	REMARKS
	Total protein	Albumin	Globulin	Non-protein		
Before exchange.....	5.5	4.0	1.5	2.2	0.42	
Immediately after.....	2.8	2.1	0.7	1.7	0.22	
15 minutes after.....	3.5	2.8	0.7	1.8	0.27	
2½ hours.....	4.6	3.3	1.3	1.8	0.30	Moderate shock
5 hours.....					0.19	
2d day.....	4.8	3.8	1.0	2.0	0.56	Normal
4th day.....	4.9	3.4	1.5	1.3	0.42	
6th day.....	5.4	3.7	1.7	2.1	0.49	

*Experiment 105.* (See table 8). 90 per cent exchange.

*Dog 18-68.* Young female bull dog. Weight 15.3 pounds.

*November 21, 1917.* Under ether anesthesia 623 cc. of blood were withdrawn from the right femoral artery. Simultaneously 623 cc. of blood corpuscle suspension were injected into the right femoral vein. The duration of the exchange was 9 minutes. The animal showed moderate depression and slight decrease in pulse volume for several hours.

Tables 9 and 10 give figures to show the low level of serum proteins which may be effected by very large blood volume exchanges (159 and 195 per cent). The usual normal dog will not tolerate such large exchanges without exhibiting profound and often fatal shock. These two dogs were unusually resistant to this experimental procedure and give us the opportunity to study the reaction following such large



exchanges uncomplicated by shock or notable hemolysis. The low level of total proteins is to be expected and one experiment (table 10) reaches the minimum figure for total protein (0.9 per cent). We have no observation in any of our experiments to show a lower level of protein in the blood stream. The protein regeneration is very rapid in the 15-minute period as well as in the following 24 hours, indicating considerable emergency reserve material.

TABLE 9

*159 per cent blood volume exchange; dog 17-215; experiment 67*

TIME	BLOOD SERUM READINGS IN PER CENT				REMARKS
	Total protein	Albumin	Globulin	Non-protein	
Before exchange.....	6.2	3.6	2.6	1.7	
Immediately after.....	1.3	0.3	1.0	1.6	Slight hemolysis
15 minutes after.....	2.2	1.2	1.0	1.5	Slight hemolysis. Slight shock
2d day.....	4.1	2.7	1.4	1.4	Normal
3d day.....	4.3	2.1	2.2	2.0	
4th day.....	5.2	2.9	2.3	1.7	

TABLE 10

*195 per cent blood volume exchange; dog 17-232; experiment 70*

TIME	BLOOD SERUM READINGS IN PER CENT				REMARKS
	Total protein	Albumin	Globulin	Non-protein	
Before exchange.....	6.3	3.7	1.6	1.9	
Immediately after.....	0.9	0.3	0.6	1.5	
15 minutes after.....	1.4	0.7	0.7	1.7	Very slight shock
2d day.....	4.3	2.3	2.0	1.5	Normal
3d day.....	5.5	3.8	1.7	1.9	

*Experiment 67.* (See table 9). 159 per cent exchange.

*Dog 17-215.* Adult female fox terrier. Weight 15.25 pounds. Blood volume on July 19, 1917 (by dye method) was 858 cc.

*July 31, 1917.* Under ether anesthesia 1105 cc. of blood were withdrawn from the right femoral artery. Simultaneously 1105 cc. of blood corpuscle suspension were injected into the right femoral vein. The duration of the exchange was 10 minutes. There was slight decrease in force of pulse beat for about an hour. Animal showed little sign of depression thereafter.

*Experiment 70.* (See table 10). 195 per cent exchange.

*Dog 17-232.* Young female coach dog. Weight 13.5 pounds. Blood volume on July 19, 1917 (by dye method) was 714 cc.

August 3, 1917. Under ether anesthesia 1200 cc. blood were withdrawn from the right femoral artery. Simultaneously 1200 cc. of blood corpuscle suspension were injected into the right femoral vein. The duration of the exchange was 10 minutes. The pulse pressure was poor for about an hour following the exchange.

TABLE 11

PERCENTAGE EXCHANGE	EXPERIMENT NUMBER	TOTAL PROTEIN				TOTAL DROP IN PROTEIN	PER CENT PROTEIN REGAINED		CLINICAL SHOCK	HEMATOCRIT (PER CENT BLOOD CELLS)			
		Before exchange	At end of exchange	15 minutes after exchange	Second day		In 15 minutes	In 24 hours		Before exchange	At end of exchange	15 minutes after exchange	Second day
67	60	5.3	2.8	3.9		2.5	1.1		0				
74	92	5.5	3.7	3.8		1.8	0.1		0	50	58	58	
74	87	6.2	3.3	3.9		2.9	0.6		+++	58	58	55	
75	94	5.4	3.0	3.7	4.9	2.4	0.7	1.9	0	40	54	54	
75	90	5.3	2.9	3.4	4.3	2.4	0.5	1.4	++	48	54	55	38
80	84	5.6	3.1	3.1	6.6	2.5	0.0	3.5	+	50	63	58	33
80	82	5.6	2.4		4.4	3.2		2.0	0		50		44
84	74	4.9	2.6			2.3			*				
89	101	5.5	2.1	2.4		3.4	0.2		+++	60	41	53	
90	105	5.5	2.8	3.5	4.8	2.7	0.7	2.0	++	43	49	52	
91	103	6.2	3.2	3.8	4.3	3.0	0.6	1.1	++	49	49	56	35
94	62	4.9	1.8	2.8	4.8	3.1	1.0	3.0	++				
96	61	5.5	2.7	3.6		2.8	0.9		0				
98	64	6.3	3.0	2.8		3.3	-0.2		+				
99	81	4.5	1.2	2.5	4.1	3.3	1.3	2.9	++				
99	93	4.5	2.0	3.3	4.0	2.5	1.3	2.0	+	58	58	58	42
100	100	5.6	3.0	2.9	4.6	2.6	-0.1	1.6	+	46	62	64	36
Averages		5.4	2.7	3.3	4.8	2.7	0.6	2.1		50	55	56	38

Shock readings: + means slight shock; ++ means moderate to severe shock; +++ means lethal shock.

\* Death from overdose of ether.

A summary of certain factors in many plasma depletion experiments will be found in tables 11 and 12 and the average figures give much interesting information. The averages of the experiments which show 100 per cent or less of blood volume exchange (table 11) show an identical emergency increase in the blood serum proteins. The two tables are practically in accord and we note that the average replacement of serum protein during the 15 minutes following the plasmapheresis

amounts to 0.5 to 0.7 per cent protein—which is an increase of 10 to 14 per cent of the total proteins. The increase during the 24 hours following the plasma depletion is considerable and amounts to 2.0 per cent protein which is an increase of 40 per cent total protein, figuring 5.0 protein per cent as the normal for a healthy dog.

Further analysis of the blood cell hematocrit figures is of interest. It is unfortunate that we did not obtain hematocrit readings in all our experiments. It is clear that the normal hematocrit before the ex-

TABLE 12

PERCENTAGE EXCHANGE	EXPERIMENT NUMBER	TOTAL PROTEIN				TOTAL DROP IN PROTEIN	PER CENT PROTEIN REGAINED		CLINICAL SHOCK	HEMATOCRIT (PER CENT BLOOD CELLS)			
		Before exchange	At end of exchange	15 minutes after exchange	Second day		In 15 minutes	In 24 hours		Before exchange	At end of exchange	15 minutes after exchange	Second day
102	80	5.6	2.6	2.8		3.0	0.2		+++				
104	96	6.4	2.1	3.2	4.5	4.3	1.1	2.4	+	52	66		38
108	98	6.3	2.2	2.7		4.1	0.5		+++	33	44	53	
109	89	5.0	2.5	3.0	5.1	2.5	0.5	2.6	+	50	60	73	41
110	83	5.8	2.3	2.8	4.2	3.5	0.5	1.9	+				46
118	63	7.1	4.2		4.8	2.9		-0.1	+				
122	104	5.7	1.9	2.3		3.8	0.4		+++				
131	66	5.7	1.0	3.1	4.0	4.1	1.5	2.4	++	47	35	38	30
141	68	5.5	2.6	2.5	3.9	2.9	-0.1	1.3	0				
159	67	6.2	1.3	2.2	4.1	4.9	0.9	2.8	+				
170	69	5.6	2.0	2.9	4.2	3.6	0.9	2.2	++				
191	77	5.9	0.9	1.2		5.0	0.3		+++				
195	70	5.3	0.9	1.4	4.3	4.4	0.5	3.4	0				
Averages		5.8	2.0	2.5	4.3	3.8	0.6	2.1		46	51	55	39

*Shock readings:* + means slight shock; ++ means moderate to severe shock; +++ means lethal shock.

periment is approximately 50, which is an indication that healthy dogs were used. There is a slight increase in the hematocrit figures at the end of the blood exchange but only to 55 per cent. This assures us that a suitable number of red cells was introduced in the red cell Locke's solution mixture which replaced the blood. There is a trifling increase in the average hematocrit figures for the 15-minute sample but only to 56 per cent, which may indicate a very slight blood concentration due to loss of Locke's solution from the circulation.

There is a distinct fall in hematocrit during the 24 hours following the plasmapheresis—an average of 7 to 12 per cent below the initial figure. This probably indicates a true loss of red cells as we must recall the fact that these red corpuscles which are introduced have

TABLE 13

PERCENTAGE EXCHANGE	EXPERIMENT NUMBER	FIBRIN IN PER CENT				TOTAL DROP IN FIBRIN	PER CENT FIBRIN REGAINED		CLINICAL SHOCK	HEMATOCRIT (PER CENT BLOOD CELLS)			
		Before exchange	At end of ex- change	15 minutes after	Second day		In 15 minutes	In 24 hours		Before exchange	At end of ex- change	15 minutes after exchange	Second day
74	92	0.21	0.16	0.15	0.28	0.05	-0.01	0.12	0	50	58	58	
74	87	0.18	0.08	0.15		0.10	0.07		+++	58	58	55	
75	94	0.21	0.19	0.23	0.44	0.02	0.03	0.25	0	40	48	54	
75	90	0.25	0.15	0.20	0.35	0.10	0.05	0.20	++	48	54	55	38
80	84	0.17	0.06	0.03	0.40	0.11	-0.03	0.34	+	50	63	58	33
80	82	0.17	0.08	0.13	0.33	0.09	0.05	0.25	0		50		44
84	74	0.36	0.15			0.21			*				
89	101	0.24	0.11	0.11		0.13	0.00		+++	60	41	53	
90	105	0.42	0.22	0.27	0.56	0.20	0.05	0.34	++	43	49	52	
91	103	0.42	0.19	0.21	0.48	0.23	0.02	0.29	++	49	49	56	35
99	93	0.26	0.16	0.15	0.30	0.10	-0.01	0.14	+	58	58	58	42
99	81	0.32		0.19					++				
100	100	0.25	0.16	0.16	0.45	0.09	0.00	0.29	+	46	62	64	36
102	80	0.42		0.26					+++				
104	96	0.50	0.13	0.14	0.40	0.37	0.01	0.27	+	52	66		38
108	98	0.25	0.12	0.11		0.13	-0.01		+++	33	44	53	
109	89	0.35	0.12	0.11		0.23	-0.01		+	50	60	73	41
110	83	0.75	0.18	0.30	0.44	0.57	0.12	0.26	+				46
122	104	0.50	0.13	0.14	0.40	0.37	0.01	0.27	+++	52	66		38
191	77	0.44	0.13	0.10		0.31	-0.03		+++				
Aver- ages..		0.33	0.14	0.17	0.40	0.19	0.02	0.25		49	55	57	39

*Shock readings:* + means slight shock; ++ means moderate to severe shock; +++ means lethal shock.

\* Death from overdose of ether.

been submitted to considerable manipulation in the necessary washing previous to the injection. Dogs' corpuscles too are notoriously fragile. We may assume for the present at any rate that many of the red cells which were introduced had been seriously injured and went to

pieces in the blood stream during the 24 hours following the blood exchange.

We can review the *fibrin analyses* in table 13 and at once a decided difference appears when we compare the serum protein curve with that of the plasma globulin, fibrinogen. The exchange of blood reduces the fibrin content to about the same level—that is, we can wash out the same percentage of fibrinogen by the usual plasmapheresis as we do in the case of the serum proteins. The fibrin content is reduced to a little less than one-half normal—from 0.33 to 0.14. During the 15 minutes following the plasma depletion there is no emergency reaction on the part of the fibrin as is so constant for the serum proteins. During the next 24 hours the fibrin is restored completely to normal. This may mean that there is no emergency reserve of the fibrin as it can be produced so rapidly in the body in any emergency. We know of many other facts which point to *complete dissociation* of fibrin and other blood proteins as to production and repair and general usefulness in the body economy.

#### DISCUSSION

A theoretical consideration of the factors involved in this protein replacement is difficult at this time. It may be claimed that this increase represents, in part at least, not a true increase in the quantity of circulating serum protein, but is the result of the escape from the circulation of fluid poor in protein material. However, if any considerable escape of fluid from the circulation were to occur, one would expect to note a rise in the percentage of red cells in the circulating medium. That no sufficient change in the cell-plasma ratio does occur can be seen from the hematocrit figures given in tables 11 and 12. On the other hand, it may be that the figures represent an actual influx of protein into the circulating medium. Such an influx could conceivably come from some tissue or organ which serves as a storehouse for this type of protein material. Seitz (7) thinks that the liver acts as such a storehouse. Earlier work in this laboratory by Kerr, Hurwitz and Whipple (1) shows a lack of reserve production of serum proteins after plasmapheresis in the Eck fistula dog. This indicates that liver insufficiency may impair the normal emergency reproduction of blood proteins.

Of particular interest is the rather remarkable increase in the 15 minutes immediately following the end of the experimental depletion.

This, if blood volume changes be excluded, appears to be truly a throwing in of ready-formed materials.

While the depletion curve of the fibrin fraction of the plasma proteins brought about by our experimental procedure compares closely with the curve of depletion of the serum proteins, still a distinctly different type of curve of fibrin repletion is revealed. A fairly typical experiment is presented in table 8. This point may also be studied by an examination of the results given in table 13. In these tables it may be seen that the rapid rise immediately following the procedure of depletion which is typical of the serum proteins is absent or at least negligible in the case of fibrin. However, the body seems to be able to supply large amounts of this protein in a space of 24 hours, for as the summary in table 13 shows, the fibrin on the day following the exchange is already as high as the original figure, or, as occurs in some cases, even higher. When such an over-production does occur the level usually returns to normal in one to two days.

This lack of correspondence between the regeneration figures for serum proteins and for fibrin protein in the period of initial regeneration, we believe furnishes additional evidence against a theory which would account for all changes in protein concentration in this period by the loss from the circulation of fluids poor in protein. For, in such a case, the concentration of the proteins might be expected to occur to practically the same degree in each. That this does not occur tends to strengthen the evidence given by the hematocrit figures.

It may be pointed out that the curve of serum protein regeneration is very different for this type of experiment when compared to the experiments of Kerr, Hurwitz and Whipple. We believe that these differences are to be explained wholly by the differences in the experimental depletion of the serum protein. Kerr, Hurwitz and Whipple used *interval depletions* of smaller amounts but repeated many times during a single day. In this manner they undoubtedly removed much of the large emergency reserve which is so conspicuous in the 24-hour regeneration in the experiments tabulated above. Therefore Kerr, Hurwitz and Whipple observed a curve of protein regeneration which was much more prolonged before a return to normal was observed. These experiments supplement the earlier ones and strengthen their conclusions.

## SUMMARY

A rapid depletion of serum proteins is brought about in these experiments by the introduction of normal red blood cells suspended in a modified Locke's solution, care being taken to keep equivalent the volume of blood removed from the artery and the volume of red blood cell suspension simultaneously injected into the vein.

The serum protein depletion is roughly proportional to the size of this exchange and it is noteworthy that the rapid depletion of the total serum proteins can rarely be carried below 1.0 per cent without causing a fatal reaction.

An increase in serum protein concentration (serum protein replacement) begins immediately following the exchange or plasmapheresis. The increase is very rapid during the first 15 minutes following the exchange. The increase in serum proteins is more gradual thereafter during the first 24 hours and still more sluggish during the next few days. The normal level may be reached in 2 to 7 days.

The rapid replacement of serum proteins during the first 15 minutes following the exchange indicates some reserve supply of this material perhaps held in the body cells. The emergency supply is evidently small and the production of other similar material is difficult and requires time.

The blood fibrin reacts in a different fashion. The same initial fall is not followed by a rapid rise in the first 15 minutes. The recovery however is complete within 24 hours and probably earlier than this. Fibrin is a very labile protein as compared with the serum albumin and globulin.

Blood volume fluctuations are probably very little concerned in these experimental results. The red blood cell hematocrit ratio shows but little change during the period of initial reaction.

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## II. SHOCK AS A MANIFESTATION OF TISSUE INJURY FOLLOWING RAPID PLASMA PROTEIN DEPLETION

### THE STABILIZING VALUE OF PLASMA PROTEINS

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In the preceding communication we have established the curve of serum protein regeneration following a single rapid replacement of whole blood with a red cell Locke's solution mixture. This plasma depletion (plasmapheresis) washes out more or less of the blood proteins and lowers the concentration of the blood proteins in the circulating blood. In the preceding article we have submitted many experiments which are associated with little or no "shock," using this term in the familiar clinical sense. In this paper we wish to discuss more particularly those cases which are associated with severe or lethal shock. A number of such experiments are given in detail below. This intoxication associated with plasmapheresis has been noted by the earlier workers: Morawitz (1), Abel, Rowntree and Turner (2) and Kerr, Hurwitz and Whipple (3). A variety of explanations has been given.

The *physiological value of the serum proteins* is admittedly little understood and we believe our experiments throw some light on this point. Published work from this laboratory (3) indicates that the serum proteins cannot be concerned with the nutrition of the body cells and the constant exchange between food protein and body protein. The experiments outlined below suggest rather strongly that one important function of these proteins is their "stabilizing value."

The *stabilizing value* of the blood serum proteins is brought out with especial emphasis by two experiments (tables 19 and 20). The dog is bled large amounts from the femoral artery while simultaneously equal amounts of a washed red cell, dialyzed serum mixture are injected into the femoral vein. No shock followed an exchange of



large size which would surely have been fatal if the dialyzed serum had been replaced by Locke's solution as in the standard plasma-phoresis. During the dialysis of the serum it underwent considerable dilution while the dialyzable substances were being removed, but this dilute dialyzed serum was still able to protect the body cells against the shock which develops if the blood proteins are too much diluted as in the routine plasma depletion. We believe that this furnishes the last bit of evidence to show that the blood serum proteins make up an essential part of the environmental complex of the body cells. Too great a dilution of these substances invariably results in profound injury of certain cells and a reaction identical with "clinical shock."

When these protein substances are suddenly washed out of the blood serum there is a certain amount of similar material thrown in as an emergency reserve. If the depletion is too severe the body cells are injured by the very persistence of this abnormal condition and the condition of "shock" supervenes. Further it is evident that certain body cells are more sensitive than others to changes in the serum protein content—for example, liver cells. That these facts have some significance in relation to the general problem of clinical shock is at once evident.

Other experiments (3) already cited give proof that the simple plasma depletion with more or less clinical shock is associated with a certain amount of cell injury, as shown by the rise in urinary nitrogen in the two days following the exchange. There is neither gross nor histological evidence of cell necrosis, but this increase in nitrogen must come from body protein. This is further evidence for actual *cell injury as an essential part of the clinical complex named "shock."*

It may be noted also that when once the clinical picture of "shock" is established in these experiments we have been unable to save the animal by any of the familiar clinical measures, even by infusion of whole blood. The *essential injury* in these experiments is *cell protoplasm injury* induced by a sudden change in the colloidal solution which forms the normal environment of these cells. This may be a new type of cell injury but it may help us to understand the more complex cell injury which is probably responsible for "surgical shock."

## EXPERIMENTAL OBSERVATIONS

The various experimental methods have been described in detail in the preceding communication. To save repetition we may refer to some of the experiments detailed in the first paper of this series. The experiments given below are only types which illustrate a characteristic reaction and usually represent groups of similar experiments.

The first two tabulated experiments (tables 14 and 15) illustrate the reaction which was so common in the experiments of paper I of this series. In addition these two experiments done on the same dog at an interval of three weeks show that this procedure (plasmapheresis) does not sensitize a dog to any subsequent repetition of this procedure. This shock so exactly resembles the anaphylactic shock in dogs that it seemed necessary to exclude this possibility. Other experiments giving the same negative results need not be instanced.

TABLE 14

*80 per cent blood volume exchange; very slight shock; dog 18-35; experiment 84*

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	HEMATO-CRIT RED CELL PER CENT
	Total protein	Albumin	Globulin	Non-protein		
Before exchange.....	5.6	4.6	1.0	2.4	0.17	50
Immediately after.....	3.1	2.5	0.6	2.8	0.06	63
15 minutes after.....	3.1	2.9	0.2	2.6	0.03	58
4 hours.....	4.1	3.3	0.8	2.5	0.06	58
8 hours.....	6.1	4.9	1.2	2.5	0.16	45
2nd day.....	6.6	5.5	1.1	2.7	0.40	33

The curve of protein regeneration during the eight hours following this plasma depletion is beautifully shown in both experiments. The emergency reserve was sufficient to replace all the serum proteins removed (table 14) but it is noted that the total drop in serum protein was but 2.5 per cent total protein.

*Experiment 84.* (See table 14). 80 per cent exchange.

*Dog 18-35.* Female bull pup. Weight 15 pounds. Appears to be in excellent condition.

*September 12.* Under ether anesthesia 545 cc. of blood were withdrawn from the right femoral artery. Simultaneously and at the same rate 545 cc. of Locke's corpuscle suspension were injected into the right femoral vein. The exchange was effected in 7 minutes. There was a fall in rectal temperature of about 1°C. following the exchange. No definite sign of intoxication was noted except for a slight amount of vomiting 2 hours following the exchange. The animal appeared to be in good condition on the 2nd day.

*Experiment 92.* (See table 15). 74 per cent exchange.

*Dog 18-35.* Female bull pup. Weight 16.25 pounds. On September 12 an 80 per cent exchange was effected in 7 minutes without any decided signs of intoxication (see table 14).

*October 3.* Dog seems to be in excellent condition. Under ether anesthesia 545 cc. of blood were withdrawn from the left femoral artery. Simultaneously and at the same rate 545 cc. of Locke's corpuscle suspension were injected into the left femoral vein. The exchange was effected in 14 minutes. There was practically no alteration in rectal temperature and at no time were there any signs of intoxication.

*Experiment 61.* No clinical shock. 96 per cent exchange.

*Dog 18-7.* Female terrier pup. Weight 11 pounds. Estimated blood volume (by dye method) 530 cc.

*July 19.* Under ether anesthesia 480 cc. of blood were withdrawn from the right femoral artery. Simultaneously and at the same rate 480 cc. of Locke-corpuscle suspension were injected into the right femoral vein. The duration of the exchange was 12 minutes. There was practically no disturbance in rectal temper-

TABLE 15

*74 per cent blood volume exchange; no clinical shock; dog 18-35; experiment 92*

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	HEMATOCRIT RED CELL PER CENT
	Total protein	Albumin	Globulin	Non-protein		
Before exchange . . . . .	5.5	4.0	1.5	1.8	0.21	50
Immediately after . . . . .	3.7	2.7	1.0	1.7	0.16	58
15 minutes after . . . . .	3.8	2.9	0.9	1.8	0.15	58
2½ hours . . . . .	4.3	3.4	0.9	1.8	0.18	48
5½ hours . . . . .	4.6	3.5	1.1	1.8	0.21	48
2nd day . . . . .					0.28	

ature. There was a slight amount of drowsiness for several hours. Otherwise no disturbance was noted. The total serum proteins fell from the initial value of 5.5 per cent at the beginning of the exchange to a level of 2.7 per cent at the end of the exchange. Fifteen minutes later a value 3.6 per cent was found. No samples were taken subsequently. At no time was there any decided alteration in the albumin-globulin ratio.

The next group of experiments illustrates the fatal shock which may develop following an exchange of blood equal to 100 per cent blood volume or more. From these and other experiments it is obvious that the body can supply an emergency reserve of serum proteins even during the period of profound shock which precedes death (2 to 5 hours). Moreover the ratio of albumin and globulin is not especially disturbed as is so frequently seen in severe intoxication due to bacterial invasion.

The clinical and anatomical pictures described in this condition of shock following plasma depletion are very constant and resemble in the dog the reaction observed in fatal anaphylaxis. The fall in blood pressure may be delayed several minutes—sometimes 30 minutes after completion of the exchange—but the fall in temperature is prompt. At times there may be a subsequent rise in temperature even in fatal intoxication, but often the loss of temperature control is complete and rectal temperatures of 30°C. may be recorded. Gastro-intestinal disturbance is the rule. Vomiting and diarrhea are seen early, sometimes within 30 minutes, and persist. This watery, blood-tinged diarrhea is common in fatal cases. Mucus may be very abundant in certain cases, even occasionally when recovery takes place following a severe intoxication. The dull lethargic appearance with clinical prostration is very typical of this type of shock. This picture corresponds closely with the surgical condition of "shock" associated with intoxication (for example, intestinal obstruction) or hemorrhages or prolonged operative manipulation.

The *autopsy findings* also are very uniform. For these the description of a single case will suffice. Blood removed from the heart at autopsy or from the veins at intervals before death may show delayed coagulation but this is not uniform. The fibrin content is low because this plasma protein like the serum proteins has been washed out by the exchange. The liver, spleen and kidneys show engorgement, usually most marked in spleen and liver. The thorax, heart and lungs are negative. The stomach may be pale or slightly injected. The entire small intestine shows congestion of its mucosa often more marked in the upper tract. The mucosa may be velvety, purplish red and coated with thick creamy mucous. All degrees of congestion are found. The lumen contains a thin, watery, blood-tinged fluid in which more or less mucus is present. The colon shows the same material and a mottled congested mucosa.

It will be noted that this picture of shock is almost identical with that produced by large doses of adrenalin, clamping of aorta or vena cava and trauma of the intestines, recently studied and described by Erlanger (5).

*Experiment 98.* (See table 16). 108 per cent exchange.

*Dog 18-20.* Female bull-terrier pup. Weight 19.4 pounds. On September 20 an exchange of 109 per cent in 14½ minutes produced moderately severe shock.

*October 31.* Under ether anesthesia 950 cc. of blood were withdrawn from the left femoral artery. Simultaneously and at about the same rate 1000 cc. of Locke's corpuscle suspension were injected into the left femoral vein. The duration of

the exchange was 12½ minutes. The rectal temperature fell about 1°C. during the exchange. Subsequently there was a fall of 1° more, when death occurred. The arterial tension was fairly good at the end of the exchange but became quite poor in the course of the next 15 minutes. It remained poor until death. Deep respiration developed in the course of the first hour following the exchange. No marked signs of depression or loss of power of attention appeared for about 2 hours after the exchange. The condition then became rapidly worse and death occurred 1 hour later.

*Autopsy* shows swollen congested spleen. The liver is deep red, the lobulation is obscure. The mucosa of the entire intestinal tract is congested. There is a considerable excess of mucus. The other organs are negative. Blood drawn from the heart at time of autopsy when placed in a test tube clots in 25 minutes; that which is left in contact with the tissues clots in 10 minutes. The clot formed is quite flabby.

*Experiment 101.* (See table 17). 89 per cent exchange.

*Dog 18-5.* Young male terrier. Weight 18.5 pounds. On July 18 an exchange of 67 per cent produced a very mild grade of shock.

TABLE 16

108 per cent blood volume exchange; fatal shock; dog 18-20; experiment 98

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	HEMATOCRIT RED CELL PER CENT	REMARKS
	Total protein	Albumin	Globulin	Non-protein			
Before exchange.....	6.3	4.4	1.9	2.1	0.25	33	
Immediately after.....	2.2	1.3	0.9	1.4	0.12	44	
15 minutes after.....	2.7	1.7	1.0	1.6	0.11	53	
2 hours.....	3.6	2.5	1.1	2.1	0.12	42	Death

*November 8.* Under ether anesthesia 750 cc. of blood were withdrawn from the left femoral artery. Simultaneously 750 cc. of Locke's corpuscle suspension were injected into the left femoral vein. The duration of the exchange was 11 minutes. The rectal temperature fell 2°C. as a result of the exchange but returned subsequently to the original level of slightly above 40°. The arterial tension was good at the end of the exchange but became poor within the course of 30 minutes. Definite signs of general depression or "shock" appeared within an hour following the end of the exchange. The power of attention was completely lost 2 hours later. Death occurred 5 hours after the exchange.

*Autopsy:* The thymus is somewhat larger than normal. Otherwise the thoracic organs are negative. The spleen is moderately enlarged and congested. The liver is negative except for pronounced indistinctness of lobulation. The mucosa of the duodenum is slightly reddened, but no excess of mucus is found in the lumen.

*Experiment 76.* Fatal shock. 178 per cent exchange.

*Dog 18-7.* Female terrier pup. Weight 10.5 pounds.

A 96 per cent exchange was carried out on July 19 (see exper. 61) with practically no sign of shock.

On July 26 an exchange of 118 per cent was effected with very slight reaction. Immediately following this second exchange an injection of phosphorus was given. No definite injury was noted (see exper. 65, table 24).

*August 16.* Animal appeared to be in excellent condition. Under ether anesthesia 850 cc. of blood were withdrawn from the right carotid artery. Simultaneously and at about the same rate 900 cc. of Locke's corpuscle suspension were injected into the right external jugular vein. The duration of the exchange was 7 minutes. Within a few minutes definite signs of shock appeared. The pulse rapidly diminished in volume, the respiration became irregular and the rectal temperature fell steadily from the original of 38.5°C. to 36.1° at the time of death, 1½ hours following the exchange.

*Autopsy:* The thoracic organs are negative. The spleen, liver and kidneys show moderate congestion. The upper part of the small intestines shows marked thickening and congestion of the mucosa. Thin bloody fluid is present in considerable quantities within the lumen of the intestines. The mucosa of the large intestine is slightly congested. The pancreas is decidedly swollen by interlobular edema. There is a considerable amount of hemolysis.

TABLE 17  
89 per cent blood volume exchange; fatal shock; Dog 18-5; experiment 101

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	HEMATOCRIT RED CELL PER CENT	REMARKS
	Total protein	Albumin	Globulin	Non-protein			
Before exchange.....	5.5	4.4	1.1	2.2	0.24	60	
Immediately after.....	2.1	1.7	0.4	1.7	0.11	41	
15 minutes after.....	2.4	1.8	0.6	2.2	0.11	53	
3 hours.....					0.13	54	
5 hours.....	3.6	2.6	1.0	2.2	0.21	56	Death

The refractometric estimation of serum proteins was not carried out. Nitrogen estimation by the Kjeldahl method showed that the total plasma proteins decreased as a result of the exchange from 4.9 per cent to 2.0 per cent. The fibrin content of the plasma fell from a level of 0.44 per cent to a level of 0.19 per cent as a result of the exchange. The value at the end of 15 minutes was 0.15 per cent, and 0.24 per cent at autopsy.

From a perusal of many experiments in this paper it is evident that there are wide individual variations in the susceptibility of different dogs to the plasma depletion. But each individual dog will usually react with considerable uniformity to a repeated plasmapheresis of unit volume if sufficient time is allowed between experiments for complete recovery. This is noted in tables 14 and 15 which give data from two experiments performed on the same animal at 3 weeks interval. If the second or succeeding exchanges are larger in amount

we may expect to record increasing degrees of intoxication and finally severe or fatal shock. This fact is illustrated by the preceding experiment (no. 76) in which two previous plasma depletions had no ill effects. The first one was an exchange of only 96 per cent with no signs of intoxication. The second exchange was slightly larger (118 per cent) and caused a slight intoxication. The final exchange of 178 per cent caused a prompt and fatal intoxication with the characteristic post-mortem findings described in fatal shock.

*The substitution of serum for Locke's solution in plasma depletion (fibrinphoresis)*

*Experiment 323.* (See table 18). 144 per cent exchange.

*Dog 19-74.* Adult female mongrel terrier. Weight 16 pounds.

*August 2.* Under ether anesthesia 1050 cc. of blood were withdrawn from the right femoral artery. Simultaneously and at the same rate 1100 cc. of a serum corpuscle mixture were injected into the right femoral vein. This serum corpuscle mixture consisted of 550 cc. of packed dog corpuscles washed twice with sterile

TABLE 18

*144 per cent blood volume exchange; substitution of serum for Locke's solution in plasma depletion; no clinical shock; dog 19-74; experiment 323*

TIME	TOTAL SERUM PROTEINS PER CENT	HEMATOCRIT RED CELL PER CENT	FIBRIN IN PER CENT
Before exchange . . . . .	6.3	58	0.60
Immediately after . . . . .	5.4	49	0.08
15 minutes after . . . . .	5.8	54	
3 hours . . . . .		50	0.30
24 hours . . . . .	5.3	45	0.50

calcium-free Locke's solution in the customary way, to which was added an equal amount of serum. The serum for this purpose was obtained by drawing into large centrifuge tubes blood from normal dogs. After the process of clotting was completed the tubes were centrifugalized and the supernatant serum withdrawn. The duration of the exchange was 6 minutes. Ether anesthesia lasted 1 hour. The rectal temperature fell  $2\frac{1}{4}^{\circ}\text{C}$ . as a result of the procedure but returned to the original level within the space of about 2 hours. The arterial tension was good at the end of the exchange. There was at no time any definite impairment in the quality of the pulse. The animal showed no signs of shock.

*August 3.* Dog in excellent condition.

*The substitution of serum for Locke's solution in the plasma depletion (fibrinphoresis)*

*Experiment 324.* 176 per cent exchange. Female bull-terrier pup (3 months old). Weight 15 pounds.

*August 5.* Under ether anesthesia 1200 cc. of blood were withdrawn from the right femoral artery. Simultaneously and at the same rate 1275 cc. of serum corpuscle suspension made up as described in experiment 323 were injected into the right femoral vein. The duration of the exchange was 15 minutes. Ether

anesthesia lasted 40 minutes. The rectal temperature was depressed about 3°C. for a period of about 2 hours. The animal remained quiet for a period of 1 hour following the exchange, the power of attention being, however, good at all times. At the end of this time the animal was in excellent condition. The hematocrit values fluctuated but slightly as a result of the experimental exchange. The fibrin content of the plasma fell from its normal level of 0.43 to 0.30 per cent 3 hours after the exchange. The reading after 24 hours was 0.65 per cent.

The two preceding experiments (table 18, expts. 323 and 324) bring out several important facts. The experimental manipulation of the red cells and the actual exchange of one mass of red cells for another are not responsible for the intoxication. In these two experiments we employed washed red cells from normal dogs prepared exactly as described for other experiments. These cells were suspended *not in Locke's solution* but in the proper amount of *fresh normal dog serum*. These large exchanges then did not wash out any serum proteins but did remove much of the fibrin. These experiments serve as good controls of the operative procedures. These large exchanges gave no evidence of any resultant intoxication. The last one especially (exper. 324) was a particularly large exchange (176 per cent) and done upon a young dog. Our experience shows that young animals as compared with adults are more sensitive to the shock of plasma depletion.

*One hundred and fifty per cent exchange using washed corpuscles suspended in dialyzed serum*

*Experiment 327. (See table 19).*

One thousand cubic centimeters of blood were drawn from normal dogs, poured immediately into large centrifuge tubes and allowed to clot. The clot formed in each tube was freed from the side of the tube and the tube centrifugalized. The supernatant serum was removed. Three hundred cubic centimeters of this serum were then placed in 15 celloidin sacs which were then immersed in 5,000 cc. of Locke's solution containing no calcium or glucose and made about 10 per cent more concentrated than normal in order that the increased osmotic pressure might in part overcome the tendency of the serum proteins to dilute themselves by attraction of water from the surrounding fluid. After dialysis had proceeded for 4 hours the modified Locke's solution was replaced by 10,000 cc. more of fresh solution of the same constitution. Dialysis was then continued for 11 hours, at the end of which time the serum contained in the celloidin sacs had increased from 300 cc. to 450 cc. To 400 cc. of the dialyzed serum 600 cc. of dog corpuscles twice washed with calcium-free Locke's solution in the ordinary way were added. The mixture was strained and heated to 38°C.

Under ether anesthesia the entire 1000 cc. of the serum corpuscle mixture were injected into the right femoral vein of a normal short-haired bull pup weighing



12.5 pounds. Simultaneously and with moderate fluctuations in the rate of flow, 850 cc. of blood were withdrawn from the right femoral artery. Thirty-five minutes were consumed in effecting the exchange. The animal showed but little alteration in body temperature as a result of the exchange. Consciousness returned shortly after the discontinuance of the anesthetic. The animal was somewhat quiet for a period of about 45 minutes. Subsequently he was bright and apparently in very good condition.

*One hundred and ninety-nine per cent exchange using washed corpuscles suspended in dialyzed serum*

*Experiment 329.* (See table 20). Nine hundred cubic centimeters of blood were drawn from normal dogs, poured immediately into large centrifuge tubes and allowed to clot. The clot formed in each tube was freed from the side of the tube and the tube centrifugalized. The supernatant serum was removed. Three hundred and fifty cubic centimeters of this serum were then placed in 18 cel-

TABLE 19  
*150 per cent blood volume exchange using washed corpuscles suspended in dialyzed serum; experiment 327*

SAMPLE	BLOOD SERUM READINGS IN PER CENT			
	Total protein	Albumin	Globulin	Non-protein
Of serum of perfusate:				
Before dialysis.....	6.2	3.8	2.4	1.7
After dialysis.....	3.3	2.5	0.8	1.2
Of dog perfused:				
Before exchange.....	5.7	3.0	2.7	1.9
Immediately after.....	3.8	2.1	1.7	1.9
4 hours after.....	4.5	2.3	2.2	2.0

loidin sacs which were then immersed in 4000 cc. of Locke's solution containing no calcium or glucose, and made about 10 per cent more concentrated than normal. After dialysis had proceeded for 5 hours the modified Locke's solution was replaced by 9000 cc. of fresh modified Locke's solution. Dialysis was then continued for 10 hours, at the end of which time the serum contained within the celloidin sacs had increased from 350 cc. to 450 cc. To 400 cc. of the dialyzed serum 600 cc. of dog corpuscles twice washed in calcium-free Locke's solution in the ordinary manner, were added. The mixture was strained and warmed to 38°C.

A normal short-haired black female mongrel terrier (no. 20-62), weighing 10.2 pounds, was anesthetized with ether and the entire corpuscle suspension was injected into the right femoral vein. Simultaneously and at the same rate 925 cc. of blood were withdrawn from the right femoral artery. The exchange was effected in 10 minutes. The temperature fell to 34.7°C. immediately following the exchange but under the influence of the heat-pad returned to 37.5°C. within a space of about 1½ hour. The animal regained consciousness within about 30

minutes following the exchange and was rather quiet for another 30 minutes, but thereafter appeared to be quite normal. The pulse was at no time markedly depressed.

The two experiments, tables 19 and 20, confirm the two preceding experiments (table 18) using fresh dog's serum. Suspension of washed red blood cells in fresh dialyzed dog serum (tables 19 and 20) gives a mixture which can be used in almost unlimited amounts to exchange with whole blood by the method adopted. This exchange is associated with no clinical shock. There is a slight lowering in the concentration of blood serum protein and of course the fibrinogen is almost completely washed out of the blood. This fibrinogen, however, can be reproduced rapidly and gives no clinical reaction as its normal content is reestablished in the blood in a few hours.

TABLE 20

*100 per cent blood volume exchange using washed corpuscles suspended in dialyzed serum; experiment 329*

SAMPLE	BLOOD SERUM READINGS IN PER CENT				HEMATO-CRIT RED CELL PER CENT	UREA NITROGEN PER 100 cc.	NON-PROTEIN NITROGEN PER 100 cc.
	Total protein	Albumin	Globulin	Non-protein			
Serum of perfusate:						mgm.	mgm.
Before dialysis.....	6.7	4.0	2.7	1.5		20	40
After dialysis.....	5.1	3.5	1.6	1.0		2	16
Of dog perfused:							
Before exchange.....	5.9	4.3	1.6	1.6	45.1		
Immediately after....	4.9	3.5	1.4	1.6	57.4		

It appears from these experiments that the essential factor responsible for the "shock" is the dilution of the serum proteins which is effected by the plasma depletion. The body cells cannot tolerate this diluted medium which for them is an abnormal environment. Protoplasmic injury is readily proved and if this injury is too extensive we note a familiar sequence of events which ends with fatal "shock." One may point out the narrow line which delimits a mild injury due to this plasma dilution from a severe or lethal injury and at times the reaction almost approaches the "all or none law." The change in urinary nitrogen following a moderate reaction and plasma depletion may be almost zero but following a severe or almost fatal shock due to plasma depletion we may observe a rise in urinary nitrogen on the day following which amounts to 100 to 200 per cent increase over nor-

mal. This indicates a serious injury of protein substance in the body. In a fatal plasmapheresis we may note a rapid increase in the blood non-protein nitrogen which may show over 100 per cent rise within 3 to 4 hours.

#### PLASMAPHARESIS COMPLICATED BY KNOWN TISSUE INJURY

In the large table 21 are collected a number of experiments to show that the presence of *injured liver cells* will predispose an animal to severe or lethal shock following a control or standard plasmapheresis. The control experiments show little or no shock following the plasma depletion of a given volume. But the same exchange performed after chloroform or phosphorus usually results in fatal shock. These experiments are in contrast to those in table 28, which presents the results of plasma depletion associated with cell injury of the kidney, pancreas and intestine. Injured cells of these organs do not modify the reaction following a standard plasmapheresis.

The three following experiments (tables 22, 23 and 24) illustrate in detail the reaction which follows plasmapheresis when preceded by chloroform anesthesia to insure a certain amount of liver necrosis and injury. The first of this group (table 22) gives a control plasmapheresis to prove that the plasma depletion alone was not responsible. The amount of liver injury was not extreme and could be tolerated by any normal animal with no clinical reaction. Note other experiments with controls in table 21.

The emergency reaction which makes possible a rapid replacement of the washed out serum proteins shows in all these experiments. The presence of the injured liver and the development of fatal shock does not modify the usual reaction by which a considerable amount of serum proteins is thrown into the circulation. This may suggest that this reaction is not purely a functional reflex but perhaps a physical phenomenon in which we see a simple exchange of protein between body cells and the circulating blood plasma—a simple washing out of a given substance related to the serum proteins which is normally present in certain body cells.

TABLE 21

*Liver injury predisposes to fatal shock after plasma depletion*

DOG NUMBER	POISON	BLOOD VOLUME EX- CHANGE		SHOCK	BLOOD SERUM PROTEINS IN PER CENT				REMARKS
		Per cent	Time in minutes		Before exchange	End of exchange	15 minutes after	24 hours after	
17-212	0	141	12	None	5.5	2.6	2.5	3.9	
17-212	Chloroform (1 hour)	144	13	Fatal	5.5	2.1	3.4		
18-6	0	118	17	Slight	7.1	4.2		4.8	
18-6	Chloroform (1½ hour)	175	12	Fatal	6.6	1.7	2.5		
18-9	0	170	12	Moderate	5.6	2.0	2.9	4.2	
18-9	Chloroform (1½ hour)	198	10	Severe	4.8	2.0	2.4	4.0	Drug given 48 hours previ- ously
17-215	0	159	10	Slight	6.2	1.3	2.2	4.1	
17-215	Phosphorus (17.5 mgm.)	140	7½	Fatal	4.9	1.2	2.1		Drug given 40 hours previ- ously
17-233	0	67	5½	Slight					
17-233	Phosphorus (14 mgm.)	118	15	Fatal	4.9	0.7	2.1		
18-34	0	75	6	Moderate	5.3	2.9	3.4	4.3	
18-34	Phosphorus (5.2 mgm.)	77	6½	Fatal	5.6	3.1	3.5		
18-7	0	96	12	None	5.5	2.7	3.6		
18-7	Phosphorus (11 mgm.)	118	7	Moderate	6.5	1.4	3.2	3.7	Drug given 5 hours later
18-66	0	91	9	Moderate	6.2	3.2	3.8	4.3	
18-66	Hydrazine (140 mgm.)	82	9	Fatal	5.7	4.2			
18-68	0	90	9	Moderate	5.5	2.8	3.5	4.8	
18-68	Hydrazine (100 mgm.)	88	9	Moderate	4.9	2.6	3.3	4.6	
18-68	Hydrazine (100 mgm.)	95	7	None	5.2	2.3	3.1		

Poison given in every experiment 18 to 26 hours before plasmapheresis unless otherwise noted.

*Plasmapheresis before and after chloroform*

*Experiment 71.* (See table 22). 198 per cent exchange.

*Dog 18-9.* Female bull-terrier pup. Weight 12 pounds.

*August 2.* Plasmapheresis, 170 per cent exchange in 12 minutes. Little if any intoxication.

*August 4.* Chloroform anesthesia for 1½ hour, undergoing recovery without clinical signs of injury.

*August 6.* Animal appears to be in excellent condition. Under ether anesthesia 1081 cc. of blood were withdrawn from the left femoral artery. At the same time and at the same rate 1081 cc. of Locke's corpuscle suspension were injected into the left femoral vein. The exchange was effected in a space of 10 minutes. There was a steady fall in blood pressure and in the volume of the pulse. An extreme grade of depression was present within a half-hour and the heart beat was barely palpable 3 hours after the exchange. The rectal temperature had fallen at this time to a level of 30°C. From this point on slow but gradual improvement was noted. Eventually complete recovery occurred.

TABLE 22

*198 per cent blood volume exchange; plasmapheresis following chloroform; dog 18-9; experiment 71*

TIME	BLOOD SERUM READINGS IN PER CENT				REMARKS
	Total protein	Albumin	Globulin	Non-protein	
Before exchange.....	4.8	3.2	1.6	1.7	
Immediately after.....	2.0	1.2	0.8	1.3	
15 minutes after.....	2.4	1.5	0.9	1.6	Profound shock
2nd day.....	4.0	2.4	1.6	2.3	
3rd day.....	4.9	3.1	1.8	2.0	Good recovery

*Plasmapheresis following chloroform*

*Experiment 72.* (See table 23). 175 per cent exchange.

*Dog 18-6.* Young-adult female Dachshund. Weight 13.4 pounds. Blood volume on July 1 (by dye method) was 761 cc.

On July 24 a 118 per cent exchange was performed in 8 minutes with little or no shock.

*August 7.* Chloroform anesthesia for 1½ hour.

*August 8.* Under ether anesthesia 1065 cc. of blood were withdrawn from the left femoral artery. Simultaneously an equal quantity of Locke's corpuscle suspension was injected into the left femoral vein. The duration of the exchange was 12 minutes. The rectal temperature showed little immediate alteration as a result of the exchange. However a gradual fall in temperature soon appeared, the level of 36.4°C. being reached at the time of death, 1½ hour later. The arterial pulse became slow and weak almost at once following the exchange. The respiration was gasping in character within 15 minutes following the exchange

and a profound degree of depression existed. The condition gradually became worse and death occurred 1½ hour following the exchange.

*Autopsy:* The blood drawn from the heart shows no tendency to clot within the space of 24 hours. Even such blood when placed in contact with fresh tissues shows no tendency to clot. The thoracic organs are negative. The spleen is somewhat enlarged and the Malpighian bodies are approximately twice their normal size. The pancreas is slightly congested. The liver is congested. A considerable amount of necrosis due to chloroform injury is seen in the centers of the lobules. Histological examination shows a fairly extensive central hyaline necrosis involving about one-half of each liver lobule. There is some fatty de-

TABLE 23

*175 per cent blood volume exchange; plasmapheresis following chloroform; dog 18-6; experiment 78*

TIME	BLOOD SERUM READINGS IN PER CENT				REMARKS
	Total protein	Albumin	Globulin	Non-protein	
Before exchange.....	6.6	3.6	3.0	1.7	Death
Immediately after.....	1.7	0.6	1.1	1.6	
15 minutes after.....	2.5	1.5	1.0	1.6	
1½ hours after.....					

TABLE 24

*144 per cent blood volume exchange; plasmapheresis following chloroform; dog 17-212; experiment 78*

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	HEMO-GLOBIN PER CENT (SAHLI)	REMARKS
	Total protein	Albumin	Globulin	Non-protein			
Before exchange.....	5.5	3.1	2.4	1.6	0.33	97	Death
Immediately after.....	2.1	1.4	0.7	1.5	0.13	92	
15 minutes after.....	3.4	2.4	1.0	1.3	0.18	92	
2 hours.....	5.5	3.6	1.9	1.7	0.12	93	

generation of the liver cells in the mid-zone of each lobule. This lesion could be tolerated by a dog with few if any clinical symptoms. The kidneys show considerable engorgement of the medulla. The mucosa of the entire gastro-intestinal tract is pink and a considerable amount of mucus is contained in the lumen.

#### *Plasmapheresis following chloroform*

*Experiment 78.* (See table 24). 144 per cent exchange.

*Dog 17-212.* Young adult female spaniel. Weight 14.5 pounds. Blood volume on July 19 (by dye method) was 632 cc.

An exchange of 94 per cent was performed on July 23, and another of 141 per cent on August 1, with practically no signs of shock in either case.

*August 21.* Chloroform anesthesia for 1 hour.

*August 22.* Animal appears to be in excellent condition. Under ether anesthesia 950 cc. of blood were withdrawn from the left carotid artery. Simultaneously 950 cc. of Locke's corpuscle suspension were injected into the left external jugular vein. The duration of the exchange was 13 minutes. The rectal temperature gradually fell about 4°C. from the normal level in the 2 hours following the exchange. Fluid blood-stained feces were noted at the end of the first hour following the exchange. The dog went into profound shock and died 2 hours following the exchange.

*Autopsy:* The thoracic organs are negative. The spleen and kidneys show considerable congestion. The liver is large and congested. In gross there is evidence of chloroform injury and histological sections show an early stage of chloroform necrosis which involves liver cells in the centers of lobules. This injury is slight in degree and by itself would give no clinical reaction in the dog. The mucosa of the stomach and small intestines is thickened and dark red in color. A considerable excess of mucus and fluid material is present in the intestinal lumen.

The following experiment (table 25, exper. 95) is complete in that a control plasmapheresis causes only a little intoxication. The dose of phosphorus is less than one-half a lethal dose and would be tolerated by a normal dog without clinical symptoms. The combined phosphorus injury and a second plasmapheresis causes a typical lethal shock.

It may be noted that the *hematocrit* figures which are complete for this experiment show no evidences of any definite change in red cell plasma ratio. The same observation holds in the chloroform experiments. When we review all these shock experiments and compare them with duplicate experiments in which no shock appears we cannot assign any of these reactions to a process of concentration of the blood. In certain experiments there is a rise in cell hematocrit taken 15 minutes and 1 to 4 hours after the exchange. But the same rise is noted at the very end of the exchange and the correct explanation we believe is to be found in the red cell mixture introduced. This red cell mixture contains more red cells per cubic centimeter than the blood of the dog under observation. There is a constant fall of hematocrit on the 2nd day but we believe this is to be explained by the disintegration of the red cells which have been injured in the routine process of washing in Locke's solution.

The second phosphorus experiment (no. 73) is given in table 21. The control plasmapheresis caused no reaction but the same exchange

preceded by a small dose of phosphorus was fatal in 2 hours. In another experiment the plasmapheresis was followed by a large dose of phosphorus. The intent was to follow the curve of protein regeneration as influenced by this drug which causes such characteristic liver injury.

*Plasmapheresis following phosphorus*

*Experiment 95.* (See table 25). 77 per cent exchange.

*Dog 18-34.* Female bull pup. Weight 17.1 pounds. Blood volume (by dye method) was 805 cc.

*September 5.* The usual plasmapheresis with 80 per cent exchange was completed in 4 minutes without the production of shock.

*September 26.* Plasmapheresis with 75 per cent exchange was carried out in 6 minutes. There was a certain amount of clinical depression, but no serious shock.

TABLE 25

*77 per cent blood volume exchange; plasmapheresis following phosphorus; dog 18-34; experiment 95*

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	HEMATOCRIT RED CELL PER CENT	REMARKS
	Total protein	Albumin	Globulin	Non-protein			
Before exchange.....	5.6	4.2	1.4	2.0	0.16	55	
Immediately after....	3.1	2.3	0.8	1.3	0.26	54	
15 minutes after.....	3.5	2.6	0.9	1.6	0.33	58	
3 hours.....	3.6	2.6	1.0	1.7	0.30	54	
6 hours.....	4.0	2.9	1.1	1.5	0.29	54	Profound shock

*October 16.* Phosphorus, 5.2 mgm. in olive oil, given subcutaneously.

*October 17.* Animal appears to be in excellent condition. Under ether anesthesia 600 cc. of blood were withdrawn from the right carotid artery. Simultaneously 600 cc. of Locke's corpuscle suspension were injected into the right external jugular vein. The duration of the exchange was 6.5 minutes. The arterial tension was fair at the end of the exchange, but was very poor at the end of another half-hour. Although showing a considerable amount of prostration, the animal was conscious for several hours. The animal was in profound shock at the end of 6 hours, and was found dead 12 hours after the exchange. The body was still somewhat warm, but rigor mortis was fairly well developed.

*Autopsy:* The tissues at the root of the lungs and about the smaller bronchi within the lung are somewhat edematous. The spleen is practically normal. The liver is quite pale and slightly translucent. Its lobulation is indistinct. Kidneys show slight congestion along the cortico-medullary line. The stomach is negative. The mucosa of the small intestine is thickened and moderately congested. A considerable amount of mucus is found in the lumen.



*Histological sections:* The liver shows very little evidence of cell injury. There are a few pale nuclei, but the fatty change so common in the cell protoplasm after large doses of phosphorus is absent. A slight increase in the leucocytes in the liver capillaries is noted. Spleen, pancreas, lung and intestines are negative.

*Plasmapheresis following phosphorus*

*Experiment 73.* 140 per cent exchange.

*Dog 17-215.* Young adult female fox-terrier. Weight 17.4 pounds. Blood volume on July 19 (by dye method) was 858 cc.

*July 31.* An exchange, 159 per cent, was performed in 10 minutes, causing no definite signs of shock.

*August 7.* Phosphorus, 17.5 mgm. in olive oil, was given subcutaneously.

*August 9.* Under ether anesthesia 1105 cc. of blood were withdrawn from the left femoral artery. Simultaneously 1105 cc. of Locke's corpuscle suspension were injected into the left femoral vein. The duration of the exchange was 8 minutes. Profound depression was in evidence almost immediately. There was a very marked weakening in the pulse. Bloody feces appeared within an hour following the exchange. Death followed the exchange by 2 hours. There was a gradual fall in rectal temperature of 4°C. during the course of the experiment. The total blood serum proteins fell from 4.9 per cent to 1.2 per cent as a result of this exchange. Other figures are not available because of loss of material.

*Autopsy:* Blood drawn from the heart immediately after death does not clot even on the addition of tissue juices. The thoracic organs are negative. The spleen is dark red and enlarged to about twice the normal size. The Malpighian bodies are large, distinct and opalescent. The liver is somewhat enlarged. The centers of the hepatic lobules are dull red while the peripheral portions are yellowish. The stomach shows distention of the superficial veins and moderate engorgement of its mucosa. The mucosa of the duodenum and upper portion of the jejunum is markedly engorged. The mucosa of the lower portion of the small intestine is but slightly reddened, while the large intestine is negative. The pyramids of the kidneys are slightly engorged. A few scars are seen in the cortex.

*Histological sections:* Liver shows early changes in cell protoplasm, especially small fat droplets. This dose of phosphorus should give a severe but not lethal liver injury. The injury at this stage is very inconspicuous. There is a notable interstitial edema of the pancreas. Other organs are negative.

*Plasmapheresis followed by phosphorus*

*Experiment 65.* (See table 26). 118 per cent exchange.

*Dog 18-7.* Young adult female mongrel terrier. Weight 10.9 pounds. Blood volume (by dye method) was 530 cc.

*July 19.* An exchange, 96 per cent, performed in 12 minutes caused no shock.

*July 26.* Under ether anesthesia 583 cc. of blood were withdrawn from the femoral artery. Simultaneously 583 cc. of Locke's corpuscle suspension were

injected into the femoral vein. The duration of the exchange was 7 minutes. With the exception of a fall of about 1°C. in rectal temperature there was little obvious disturbance as a result of the exchange. About 5 hours after the exchange 11 mgm. of phosphorus dissolved in olive oil were injected subcutaneously. On the following day the animal appeared rather quiet, but not otherwise disturbed. The food was not eaten for several days and on August 1 the dog weighed 9.25 pounds. Complete recovery occurred several days later.

TABLE 26

*118 per cent blood volume exchange; plasmapheresis followed by phosphorus; dog 18-7; experiment 65*

TIME	BLOOD SERUM READINGS IN PER CENT			
	Total protein	Albumin	Globulin	Non-protein
Before exchange.....	6.5	5.7	0.8	2.0
Immediately after.....	1.4	0.3	1.1	1.5
15 minutes after.....	3.2	1.8	1.4	1.7
2nd day.....	3.7	2.6	1.1	2.5
3rd day.....	3.8	2.4	1.4	2.3
5th day.....	5.1	2.0	3.1	1.5
10th day.....	4.3	2.0	2.3	1.8

TABLE 27

*88 per cent blood volume exchange; plasmapheresis following hydrazine sulfate; dog 18-68; experiment 108*

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	HEMATO-CRIT RED CELL PER CENT
	Total protein	Albumin	Globulin	Non-protein		
Before exchange.....	4.9	3.2	1.7	1.7	0.31	27
Immediately after.....	2.6	1.7	0.9	1.6	0.26	42
15 minutes after.....	3.3	2.3	1.0	1.4	0.31	56
3½ hours.....	3.7	2.6	1.1	1.9	0.40	41
2nd day.....	4.6	3.2	1.4	1.7		
3rd day.....	4.7	3.3	1.4	1.6		33
6th day.....	5.2	2.8	2.4	2.3		

*Plasmapheresis following hydrazine sulfate*

*Experiment 108.* (See table 27). 88 per cent exchange.

*Dog 18-68.* Female mongrel bull pup. Weight 14.3 pounds.

*November 21.* An exchange of 90 per cent performed in 9 minutes caused a moderate grade of shock.

*November 27.* Hydrazine sulfate, 100 mgm., injected subcutaneously.

*November 28.* Under ether anesthesia 575 cc. of blood were withdrawn from the left femoral artery. Simultaneously 575 cc. of Locke's corpuscle suspension were injected into the left femoral vein. The duration of the exchange was 9

minutes. There were at no time any definite signs of depression. The arterial tension remained moderately good throughout. There was a fall in rectal temperature of about 1°C. during the exchange. There was, however, a prompt return—in fact to a point slightly above the original temperature for a period of several hours, after which the temperature returned to the normal level.

The preceding experiment (table 27) gives some evidence that hydrazine sulfate as a liver poison differs somewhat when compared with chloroform or phosphorus. This dog (18-68) showed no less reaction to the control plasmapheresis than to the same exchange preceded by hydrazine sulfate. In another experiment, however, (table 21, exper. 109) we see the familiar reaction with fatal shock due to a combined plasmapheresis and hydrazine poisoning. The control of the plasmapheresis showed a definite but not severe intoxication.

The preceding table (table 28) lists the reactions which follow a plasmapheresis combined with cell injuries of various other organs and tissues. The control plasma depletion on the same dog is given in each experiment. When the remarkable reaction and fatal shock were noted in the phosphorus and chloroform experiments we suspected at once that any cell injury might render the experimental animal more sensitive to the shock of plasmapheresis. The experiments in table 28, however, show that such is not the case.

The *kidney* epithelium was injured by administration subcutaneously of uranium nitrate in suitable dosage. Two experiments show identical reactions in the control plasma depletion as in the plasmapheresis following the administration of uranium nitrate. One experiment (dog 18-35) shows a fatal reaction but there are many unusual features which we cannot explain—see table 30 below for details.

*Pancreas* injury is represented by only a single experiment but this is very clean-cut. The pancreas was injured by the injection of bile into its main duct. The control exchange gives the same negative reaction as the plasma depletion preceded by the acute pancreatitis.

The *Roentgen-ray* is able to cause a specific and extensive injury to the lymphatic tissue but especially to the *epithelium* of the *small intestine* as has been shown by the work of Hall and Whipple (6). This injury and consequent intoxication develops to its maximum on the 4th day following an exposure over the abdomen. A plasmapheresis done 24 hours after X-ray exposure gives the same reaction as in the control period. This shows that even the extensive injury which in a fatal case of X-ray intoxication involves the greater part of the epithelium of the small intestine does not modify the shock of plasmapheresis. This is in striking contrast to the liver injury.

TABLE 28

*Kidney, pancreas and intestinal epithelium injury does not predispose to shock after plasma depletion*

DOG NUMBER	POISON	BLOOD VOLUME EXCHANGE		SHOCK	BLOOD SERUM PROTEINS IN PER CENT			
		Percent	Time in minutes		Before ex- change	End of ex- change	15 minutes after	24 hours after
18-35	0	74	14	None	5.5	3.7	3.8	
18-35	Uranium (5 mgm.)	72	14	Fatal	6.3	3.9	4.6	5.8
18-48	0	104	2	Slight	6.4'	2.1	3.2	4.5
18-48	Uranium (6 mgm.)	77	4	None	6.1	3.1	4.1	4.6
18-66	0	91	9	Moderate	6.2	3.2	3.8	4.3
18-66	Uranium (8 mgm.)	96	11½	Moderate	5.1	2.9	3.2	4.8
18-65	0	100	13	Slight	5.6	3.0	2.9	4.6
18-65	Pancreatitis	94	15	None	5.3	3.3	3.9	4.8
18-68	0	90	9	Moderate	5.5	2.8	3.5	4.8
18-68	X-ray* (175 M.A.M.)	90	7	Moderate	5.9	3.2	3.9	4.7
18-65	0	100	13	Slight	5.6	3.0	2.9	4.6
18-65	X-ray (200 M.A.M.)	105	9	None	5.7	2.9	3.3	4.7

Injury given in every experiment 20 to 24 hours before plasmapheresis with exception noted: \* X-ray given 45 hours before plasmapheresis.

TABLE 29

*77 per cent blood volume exchange; plasmapheresis following uranium nitrate; dog 18-48; experiment 99*

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	HEMATO- CRIT RED CELL PER CENT
	Total protein	Albumin	Globulin	Non- protein		
Before exchange . . . . .	6.1	3.4	2.7	1.7		
Immediately after . . . . .	3.1	1.9	1.2	1.4		
15 minutes after . . . . .	4.1	2.6	1.5	1.5	0.25	61
2 hours . . . . .	4.0	2.6	1.4	1.5	0.25	36
6 hours . . . . .	4.7	2.9	1.8	1.8	0.32	29
2nd day . . . . .	4.6	3.0	1.6	2.0	0.45	41
6th day . . . . .	5.2	3.4	1.8	2.2	0.42	41
8th day . . . . .	5.1	3.4	1.7	2.2	0.29	46
9th day . . . . .	5.1	3.4	1.7	2.1		
11th day . . . . .	5.2	3.5	1.7	2.4	0.33	43

*Plasmapheresis following uranium nitrate*

*Experiment 99.* (See table 29). 77 per cent exchange.

*Dog 18-48.* Female bull pup. Weight 14.3 pounds.

*October 4.* Exchange of 99 per cent performed in 12 minutes with the production of but slight grade of shock.

*October 18.* An exchange of 104 per cent in 2 minutes was performed with very little shock.

*October 31.* Uranium nitrate, 6 mgm., given subcutaneously.

*November 1.* Animal seems to be in excellent condition. Under ether anesthesia 500 cc. of blood were withdrawn from the right carotid artery. Simultaneously 500 cc. of Locke's corpuscle suspension were injected into the right external jugular vein. The duration of the exchange was 14 minutes. At no time during the experiment was there any definite sign of shock. The arterial tension was good at the end of the exchange but shortly thereafter fell slightly for a period of several hours. The rectal temperature fell nearly 2°C. during the exchange but returned to normal in the course of several hours.

*Plasmapheresis before and after uranium nitrate*

*Experiment 97.* (See table 30). 72 per cent exchange.

*Dog 18-35.* Female bull pup. Weight 16.7 pounds.

*September 12.* An exchange of 80 per cent was performed in 7 minutes, causing slight intoxication.

*October 3.* An exchange of 73 per cent was effected in 14 minutes, with no signs of intoxication.

*October 22.* An aqueous solution of 10.4 mgm. uranium nitrate was injected subcutaneously.

*October 23.* Animal appears to be in good general condition. Under ether anesthesia 545 cc. of blood were withdrawn from the left carotid artery. Simultaneously and with no great variation in rate 545 cc. of Locke's corpuscle suspension were injected into the left jugular vein. The duration of the exchange was 13 minutes. The rectal temperature fell from 39.5 to 37°C. within a space of 4 hours. Drowsiness soon appeared, the pulse diminished in volume after about 3 hours and was decidedly poor several hours later. Bloody feces were first noted about 11 hours following the exchange. The next morning the condition was worse and a considerable amount of bloody feces had been passed. The animal was suffering from convulsive attacks. The temperature was 37°C. Death occurred about 20 hours following the exchange.

*Autopsy:* The tissues are definitely jaundiced. The thoracic organs are essentially negative. The spleen is large, the edges rounded, and on section presents definite congestion. The liver shows only indistinct lobulation. The kidneys are definitely engorged. The stomach is negative except for a slight amount of engorgement of the mucosa. The intestinal mucosa is decidedly engorged with blood, the condition being more marked in the lower portion of the gut.

*Histological sections:* Kidneys show much epithelial degeneration and necrosis involving particularly the convoluted tubules. There are numerous hyaline, hemoglobin and blood casts in the collecting tubules. Other organs present nothing of interest.

The preceding experiments (tables 29 and 30) are in conflict. The first one (exper. 99) shows a negative reaction when a standard plasma depletion is combined with kidney injury due to uranium nitrate. The other experiment (exper. 97) shows a fatal reaction but it is atypical. The shock did not develop quite as usual and the dog seemed about to recover. When the shock of plasmapheresis is tolerated for 12 hours the dog usually recovers and appears normal and active within 24 hours. This dog on the day after the experiment developed convulsions and died. There was jaundice and at autopsy signs of blood destruction. The histological sections give evidence of considerable epithelial injury in the secreting tubules of the kidney. That this kidney injury played a part in the late death is highly probable but

TABLE 30

*72 per cent blood volume exchange; plasmapheresis following uranium nitrate; dog 18-35; experiment 97*

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	HEMATO-CRIT RED CELL PER CENT	REMARKS
	Total protein	Albumin	Globulin	Non-protein			
Before exchange.....	6.3	4.7	1.6	1.7	0.32	22	
Immediately after.....	3.9	3.0	0.9	1.5	0.14	25	
15 minutes after.....	4.6	3.5	1.1	1.4	0.16	42	
2½ hours.....	5.1	3.7	1.4	1.4	0.19	38	
5 hours.....	5.2	3.9	1.3	1.4		37	
2nd day.....	5.8	4.0	1.8	1.8	0.32	30	Death

it is clear that the shock of plasmapheresis was atypical. In view of the other experiments we do not attach too much importance to a single atypical experiment which appears to be at variance with the general type reaction.

*Acute pancreatitis followed by plasmapheresis*

*Experiment 102.* (See table 31). 94 per cent exchange.

*Dog 18-65.* Female bull pup. Weight 17 pounds.

*November 7.* An exchange of 100 per cent effected in 13 minutes caused a very moderate grade of shock.

*November 13.* Under ether anesthesia laparotomy was performed and 10 cc. of sterile bile injected by means of a hypodermic needle into the pancreatic duct. The wound was closed. It is known that this will cause an intense diffuse hemorrhagic pancreatitis.

*November 14.* The animal is lively and apparently in quite good condition. Under ether anesthesia 730 cc. of blood were withdrawn from the left femoral artery. Simultaneously 730 cc. of Locke's corpuscle suspension were injected into the left femoral vein. Seven minutes were consumed in effecting the exchange. At no time was there any definite evidence of intoxication. Refer to experiment 106, table 33, for autopsy.

The preceding experiment (table 31) is complete and supports the two following experiments with X-ray injury. This pancreas was severely injured by an injection of bile into the pancreatic duct. We have frequently produced an acute hemorrhagic pancreatitis in this way and the injury may be sufficient to produce lethal intoxication by itself. That extensive injury was done this pancreas is afforded by

TABLE 31

*94 per cent blood volume exchange; acute pancreatitis followed by plasmapheresis; dog 18-65; experiment 102*

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	HEMATO-CRIT RED CELL PER CENT
	Total protein	Albumin	Globulin	Non-protein		
Before exchange.....	5.3	3.6	1.7	2.2	0.49	36
Immediately after.....	3.3	2.5	0.8	1.6	0.29	54
15 minutes after.....	3.9	2.9	1.0	1.4	0.22	58
3 hours.....	4.6	3.1	1.5	1.4	0.40	53
8 hours.....	4.3	2.9	1.4	2.2	0.34	41
2nd day.....	4.8	2.9	1.9	2.2	0.29	40
3rd day.....	4.6	2.6	2.0	2.2	0.25	35
4th day.....	4.0	2.8	1.2	2.8	0.35	32
6th day.....	5.0	2.8	2.2	2.9	0.31	27
7th day.....	5.0	3.7	1.3	2.7	0.40	29

examination of the autopsy record of this dog (exper. 106, table 33 below) which shows a scarred, indurated pancreas speckled with old fat necroses. Yet these injured pancreas cells did not modify the reaction following a controlled plasma depletion.

*Plasmapheresis following sublethal X-ray exposure*

*Experiment 111.* (See table 32). 90 per cent exchange.

*Dog 18-68.* Female bull pup. Weight 14 pounds.

*November 21.* An exchange of 90 per cent effected in 9 minutes caused a very moderate grade of shock.

*December 10.* X-ray exposure over abdomen in 4 quadrants, 2 mm. aluminum filter, 175 milliamperes minutes, with 9 inch spark gap. Distance from target to skin is 10 inches.

*December 12.* Animal seems to be in excellent condition. Under ether anesthesia 575 cc. of blood were withdrawn from the left carotid artery. Simultaneously 575 cc. of Locke's corpuscle suspension were injected into the left external jugular vein. The duration of the exchange was 7 minutes. There was but a very slight amount of depression. The arterial tension was good at the end of the exchange, but became of poorer quality in the course of the next 2 hours. The animal walked about occasionally. The rectal temperature fell about 1°C. during the course of the experiment.

*Lethal dose of X-ray followed by plasmapheresis*

*Experiment 106.* (See table 33). 105 per cent exchange.

*Dog 18-65.* Female bull pup. Weight 16.8 pounds.

*November 7.* An exchange of 100 per cent effected in 13 minutes caused a very moderate grade of shock.

*November 14.* An exchange of 94 per cent following acute experimental pancreatitis produced no definite signs of intoxication (see exper. 102, table 31).

*November 21.* X-ray exposure over abdomen in 4 quadrants, 2 mm. aluminum filter, 200 milliamperes minutes, with 9 inch spark gap. Distance from target to skin is 10 inches.

*November 22.* The animal appears to be in good condition. Under ether anesthesia 800 cc. of blood were withdrawn from the right carotid artery. Simultaneously 800 cc. of Locke's corpuscle suspension were injected into the right external jugular vein. The duration of the exchange was 9 minutes. The animal showed a very slight amount of depression as a result of the exchange. The pulse remained fair throughout. There was but slight depression of the rectal temperature (1°C.).

*November 23.* The animal seems a bit weak. Has vomited material containing some intestinal worms.

*November 24.* The dog appears somewhat better.

*November 25.* Quite weak. Mucous, blood-tinged feces. Some vomiting. Refuses food.

*November 26.* Death occurred in the afternoon. Autopsy performed at once.

*Autopsy:* Thoracic organs negative. Blood drawn from the heart clots normally. Blood urea nitrogen is 32.5 mgm. per 100 cc. Spleen is small, light red, with an increase in fibrous tissue. Liver is pale and anemic. Pancreas: lower arm is hard, shrunken and scarred; the result of the pancreatitis described in experiment 102, table 31. Many old fat necroses are present. The upper arm is scarred but appears more nearly normal. Kidneys and adrenals are negative. Gastro-intestinal tract shows only a few scattered patches of congestion.

*Histological sections:* The pancreas shows extensive fibrosis—the result of the preceding acute injury. The small intestine shows much epithelial injury in its deep crypts. There is some evidence of epithelial regeneration as well as degeneration. This epithelial injury we believe to be the immediate cause of death. Other organs present nothing of interest for this experiment.

The two preceding experiments (tables 32 and 33) show the influence of X-ray injury of the body cells upon a standard plasmapheresis.



The first experiment (table 32) shows the result of a sublethal exposure to the X-rays. The reaction to the plasma depletion in a control exchange is not modified.

The second experiment (table 33) shows a reaction recently described in some detail by Hall and Whipple (6). This reaction is due

TABLE 32

*80 per cent blood volume exchange; plasmapheresis following sublethal x-ray exposure; dog 18-68; experiment 111*

TIME	BLOOD SERUM READINGS IN PER CENT				REMARKS
	Total protein	Albumin	Globulin	Non-protein	
Before exchange.....	5.9	4.2	1.7	2.2	No shock
Immediately after.....	3.2	2.6	0.6	1.7	
15 minutes after.....	3.9	3.0	0.9	1.8	
3 hours.....	4.4	3.1	1.3	1.9	
5½ hours.....	4.5	3.3	1.2	1.9	
2nd day.....	4.7	3.3	1.4	2.7	Dog normal
4th day.....	4.5	3.0	1.5	2.6	

TABLE 33

*105 per cent blood volume exchange; lethal dose of x-ray followed by plasmapheresis; dog 18-65; experiment 106*

TIME	BLOOD SERUM READINGS IN PER CENT				FIBRIN IN PER CENT	HEMATOCRIT RED CELL PER CENT	REMARKS
	Total protein	Albumin	Globulin	Non-protein			
Before exchange.....	5.7	3.7	2.0	2.1	0.45	33	Slight shock
Immediately after.....	2.9	2.3	0.6	1.7	0.22	44	
15 minutes after.....	3.3	2.6	0.7	1.7	0.27	34	
4 hours.....	4.1	2.8	1.3	1.8	0.28	37	
7 hours.....	4.2	3.4	0.8	1.5			
2nd day.....	4.7	3.5	1.2	2.1	0.47	26	X-ray intoxication
3rd day.....	3.9	2.1	1.8	3.0		25	
4th day.....	4.0	1.7	2.3	3.8	0.72	30	
5th day.....	5.9	3.0	2.9	3.1	0.95		Death

to a lethal dose of the X-ray—in this instance 200 milliamperere minutes, 90 kilo volts, given over the abdomen. Death on the 4th day with the usual blood-tinged feces and prostration is the usual reaction in these animals given a lethal exposure of the X-ray. Details of this reaction and the post-mortem findings may be found in the publica-

tion just noted. The control plasmapheresis which was done 24 hours after the X-ray exposure did not give any symptoms of intoxication and this reaction due to the plasma depletion was not modified by the presence of a great amount of injured epithelium of the small intestine. We have many experiments to show that on the second day after X-ray exposure *epithelial injury and necrosis* can be made out histologically in the small intestine. These cells will undergo rapid autolysis under a variety of conditions and it is quite remarkable that the plasmapheresis should not be modified by this great mass of injured epithelial cells.

#### DISCUSSION

In some earlier experiments, Kerr, Hurwitz and Whipple (3), it was noted that the presence of liver injury or liver cell necrosis made a given animal much more vulnerable to the injury and consequent shock which followed a given plasma depletion. Using single rapid depletion by the method described in the experiments cited above similar results were observed. A number of such experiments are given in table 21 above and it will be noted that the control experiment in every case shows little or no shock following plasmapheresis, but an identical procedure if associated with slight liver injury was almost always fatal. There is apparently little or no difference in this respect between the liver injury due to chloroform and that due to phosphorus. The liver injury due to hydrazine sulfate was not studied in a sufficient number of experiments.

■ The interesting fact stands out that a trifling injury due to phosphorus or chloroform can be tolerated by a dog with no clinical reaction. But if at this time (24 to 48 hours after administration of the chloroform or phosphorus) we perform a plasmapheresis of small volume which was previously tolerated by the same dog with little or no intoxication, we immediately precipitate severe or fatal shock. The combination of slight liver injury and a moderate exchange (plasmapheresis) will result fatally in almost all cases. How may we explain this observation? There are many possibilities but we favor the following explanation. The chloroform or phosphorus causes an injury to many liver cells and these cells are more susceptible to other injurious agents than are the normal liver cells. A sudden change in the protein content of the blood which bathes these injured cells will react more unfavorably upon them than upon the healthy and more resistant normal liver cells. These damaged (phosphorus) and then shocked (plas-

mapharesis) liver cells form substances which are taken up by the blood and carried to all the living cells of the body. If these poisonous substances are sufficient in amount we observe the development of lethal shock. We may not assume simple intensive injury and paralysis of the *liver cells alone* because it is known that the body can tolerate complete ablation of the liver cells for a period of 5 to 7 hours (7). When we produce an intensive form of shock (plasmapharesis) we may observe death within 1.5 hour. This cannot be explained by any *local reaction* limited strictly to the liver cells.

We observe in other experiments (table 28) that cell injury of other organs (kidney, pancreas and intestine) does not modify the familiar reaction following a moderate exchange. The control and poisoning experiments give similar reactions. This indicates a peculiar relation of the liver cells to the *shock reaction* associated with plasma depletion.

#### SUMMARY

Bleeding a dog from a large artery and a simultaneous replacement of a red blood cell Locke's solution mixture may be called "plasma depletion" or "plasmapharesis." This procedure will rapidly wash out large amounts of plasma proteins and cause a precipitous fall in the blood plasma protein concentration.

The reaction following such procedures may be minimal or it may be lethal. In general the larger the exchange the greater the probability of lethal shock. Repeated plasma depletions carried out at intervals of days or weeks on the same animal will give uniform reactions if the volume exchange and other experimental factors are constant.

"Plasmapharesis" may be performed with washed red cells suspended in normal dog serum or fresh dialyzed dog serum. When we replace the Locke's solution in the red cell mixture by dog serum we remove completely the toxic effect of the plasma depletion. This gives control for the experimental procedure but, more important, gives strong indication that the *blood serum proteins are stabilizing or protective factors*. They are essential environmental factors of the circulating blood in its relation to the body cells. This may be the most important function of these plasma colloids.

The presence of injured cells of the kidney, pancreas or intestine does not seriously modify the expected reaction following a uniform plasmapharesis.

The presence of *injured liver cells* (chloroform, phosphorus) *does profoundly modify the expected reaction* following a unit plasmapheresis. A fatal shock reaction is almost constant following even a moderate plasma depletion preceded by liver injury.

This would indicate that the liver cells are particularly concerned in the peculiar shock reaction which may follow plasmapheresis and lowering of the blood plasma protein values. It may be that this type of "shock" is not unlike the common "surgical shock."

The evidence in our experiments gives strong support to the theory that in "shock" there is a *primary cell injury* which *precedes* the familiar *clinical reaction*.

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### III. FACTORS CONCERNED IN THE PERFUSION OF LIVING ORGANS AND TISSUES

#### ARTIFICIAL SOLUTIONS SUBSTITUTED FOR BLOOD SERUM AND THE RESULTING INJURY TO PARENCHYMA CELLS

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These experiments were undertaken for the purpose of investigating the formation of the serum proteins in the body. The source of the serum proteins is still a mystery in spite of indirect evidence brought out by Kerr, Hurwitz and Whipple (1) to show that the *liver* is concerned in the regeneration of new serum proteins as well as in the maintenance of the normal serum protein concentration in the circulating blood. It seems too that other organs or tissues as well as the liver must be able under emergency conditions to produce certain amounts of new serum protein. It would appear that the normal wear and tear of serum proteins must be slight as these substances can be formed only with so much difficulty when the normal level has been greatly lowered.

Theoretically, perfusion of organs should offer an ideal method of solving these problems. Using a mixture of red cells and whole serum or diluted serum or modified Locke's solution, the investigator should be able to perfuse satisfactorily the various organs or combinations of organs and tissues. A simple determination of the protein values before and after such a procedure would then give the desired information and enable one to say whether a certain organ did or did not contribute any serum proteins. Information as to whether serum proteins are used by or destroyed in these organs might also be made available.

We have been able to convince ourselves that the present methods are not satisfactory to permit of the solution of this serum protein problem just outlined above. One of the conclusions which has been forced upon us is that much of the older experimentation in the field of organ perfusion is of little or no value as regards deductions made from

such experiments which postulated a living organ or organ cells. When parenchymatous organs are perfused with Locke's solution or some modification of this solution with or without red cells, we wish to suggest the probability that the research worker is dealing not with normal cells but with cells which have been injured or destroyed by contact with the perfusate. The investigator is then perfusing a dying or dead autolysing tissue or organ. Deductions drawn from such experiments must be cautious and proper allowance in every case made for this profound injury of the parenchyma cells.

The "stabilizing value" of the blood serum proteins and the injury done the various body cells by contact with diluted plasma have been emphasized in the preceding communication. We may also note the observation of Guthrie (2) to the effect that organ transplantation is a failure if the transplanted organ is washed out with normal saline before the blood flow through the organ is reestablished. The transplanted organ does not resume its function and we may assume that its cells were definitely injured by the short period of contact with the salt solution.

The gradual slowing of the perfusion flow through any given organ is a familiar observation and we believe it may be due in part to actual injury of cells, including the endothelium. The simultaneous development of edema is a part of the same general reaction.

From many of our earlier experiments we gained the impression that perfusion or plasmapheresis with the use of Locke's solution inflicted a destructive injury upon certain body cells and that this injury of protein was responsible for the fatal intoxication and death. Our conception was somewhat as follows: the initial injury might damage irreparably liver cells (or other body cells) and from these injured organ cells were derived poisonous substances (protein split products) sufficient to cause death. It has been pointed out that complete paralysis of all liver cells could not explain this phenomenon as ablation of the liver can be tolerated for 5 to 7 hours, while the shock following a large plasmapheresis may cause death within 2 hours.

Postulating the presence in the body of some poisonous substance referable to the perfusion or plasmapheresis, we have attempted in many of our perfusion experiments to demonstrate the presence of a poison in the perfusate at the end of any given experiment. In only one experiment (exper. 5) have we evidence for the presence of any poison under these conditions of perfusion. But we have some evidence (expers. 17 and 18) to show that a known poison of protein origin

added to a given non-toxic perfusate may be in part removed within 20 to 30 minutes' continuance of perfusion. This is evidence that a poison of colloid nature may be removed from the circulation in such experiments—so the absence of a poison in our perfusates does not negative the possibility of poison being formed by the injured cells and contributed to the blood stream or perfusate. We have further been able to show that an enormous dose of proteose-like, toxic material may be wholly removed from the blood stream within a period of 5 minutes after intravenous injection in a normal dog.

We have discussed the results of the experiments given below in the light of the plasmapheresis experiments given in the two preceding papers. It may be noted that these perfusion experiments were done *before* the plasma depletion experiments. The evidence which may be taken from our perfusion experiments is not as definite as that obtained in the later plasmapheresis experiments but these data are all in harmony. A most important fact is that *physiological perfusion* of organs is very difficult and *slight modification of the blood plasma* may have profound effect upon body cells.

#### DEVELOPMENT OF PERFUSION METHODS

The notion of artificial perfusion was long ago expressed by Le Gallois (3). He maintained that by artificial perfusion life might be kept up in any portion of the animal even though separated from the rest of the body. It remained, however, for other workers actually to undertake such experiments. In 1828 Kay (4) showed that artificial perfusion with blood was capable of restoring irritability to dying muscle. Artificial perfusion of kidneys was first attempted in 1849 by Löbell (5). The work of Brown-Séquard (6) done several years later showed the necessity of oxygenation of the blood used as a perfusate. The oxygenated blood was forced through the arteries by means of a syringe. In this manner he perfused various regions including the isolated head. He found that he was able in this manner to maintain certain evidences of reflex nervous activity provided the perfusion was commenced promptly after decapitation. Ludwig and Schmidt (7) in 1868 described an apparatus by means of which blood could be forced under constant pressure from a reservoir. Improvements in aeration of the perfusion medium were made by Schröder (8). Fry and Gruber (9) devised an artificial lung by means of which the aeration of the perfusate could be accomplished without interrupting the flow of blood

to the region being perfused. Although fluctuation in the pressure supplied to the perfusion medium occurred in the work of the earlier investigators using the syringe injection method, the distinct beneficial effects of such variations in pressure were first recognized by Ludwig and Schmidt (7). Fry and Gruber (9) attached the piston of a syringe supplying the arterial pressure to a motor-driven wheel thus creating by mechanical means a pulsatile pressure. Hamel (10) emphasized the need for pulsatile pressure. He devised an apparatus in which the movements of a pendulum periodically interrupted the flow of the perfusate to the tissues, thus converting a constant pressure into an intermittent one. Jacobj (11) devised an elaborate perfusion apparatus in which pulsatile pressure was created by periodic and forcible compression of a rubber balloon placed in the arterial side of the circuit. He used the principle of aerating the blood by forcing a mixture of air and venous blood through a stretch of tubing at the end of which the blood and air were separated by gravitation. In a later paper Jacobj (12) described a method by means of which the blood was aerated by perfusion through a lung in which respiration was artificially maintained. In this manner he avoided the direct mixing of the blood with the air. In 1903 Brodie (13) published an account of an apparatus which has subsequently been used by several investigators. With it he was able to perfuse an organ with the use of no other blood than that obtained from the animal itself—a considerable advantage over many of the types previously employed. To create pulsatile pressure he suggests that a fairly distensible piece of rubber tubing placed in the arterial side be rhythmically compressed by a wooden arm.

Other forms of perfusion apparatus have been described by Hoffmann (14), Richards and Drinker (15), Friedmann (16), Mandel (17) and Kingsbury (18).

Pulse pressure as a necessary factor in the mechanics has been recently reëmphasized by Hooker (19), whose apparatus was employed in our experiments. His apparatus can be adjusted in such a manner as to furnish a pulse curve identical in form to that produced in a normal intact animal. Aeration is effected.

In addition to the purely mechanical methods of perfusion another slightly different procedure has been employed by some. As far back as 1881 Martin (20) attempted to study the activity of the heart by diverting all of the blood issuing from the aorta back into the right auricle. A heart-lung preparation was thus effected, the circulation being successfully excluded from the rest of the body. This procedure



or modifications thereof have been used by many workers since that time. In 1914 Bainbridge and Evans (21) substituted this living preparation for the artificial perfusion machine. The organ to be perfused received its blood directly from the aorta of the preparation. The venous blood issuing from the vein was returned to the right side of the heart. The perfused tissue thus received blood aerated by the lungs and under pulsatile pressure supplied by the heart itself. It should be remarked that in this form of perfusion the study of the perfused organ is complicated by the metabolism of the heart and lungs themselves.

#### EXPERIMENTAL OBSERVATIONS

All perfusion experiments were performed by use of the apparatus designed and described in detail by Hooker (19), (22). Through his courtesy we were able to obtain this machine which was made after the model of his original apparatus. We take this opportunity to acknowledge our appreciation for valuable assistance on the part of Dr. D. R. Hooker. The general experimental procedures are covered by the brief description in individual experiments. All experiments were done on dogs under complete ether anesthesia. In all perfusion experiments the dog was placed upon a warm pad to keep up the body temperature.

##### *Perfusion of hind legs with Locke's solution*

*Experiment 5.* Male bull pup. Weight 4.8 kilos.

Under ether anesthesia cannulae were inserted and the hind legs were perfused for  $\frac{1}{2}$  hour with Locke's solution. The temperature of the perfusate varied between 30° and 40°C. The perfusion pressure was between 100 and 110 mm. mercury. The pulse pressure was between 20 and 30 mm. mercury. Pulse rate 130 a minute. The 240 cc. of perfusate recovered at the end of the perfusion contained 77,000 red blood cells per cubic millimeter. Hemolysis was moderate in amount.

To test the toxicity of this perfusate the cells were removed by centrifugalization and 100 cc. of the supernatant fluid were injected intravenously into a normal dog. A rise of 1.4° in temperature with vomiting and diarrhea was noted. The pulse was not markedly altered. The perfusate was therefore moderately toxic.

Ten cubic centimeters of the centrifugalized perfusate were also injected intraperitoneally into a 100 gram rat. Slight toxicity was evident.

##### *Perfusion of hind legs with red corpuscles suspended in Locke's solution*

*Experiment 7.* Male collie mongrel. Weight 16 pounds.

The hind legs were perfused with a suspension of blood corpuscles in Locke's

solution. The temperature of the perfusate varied between 32° and 38°C. The mean pressure was 50 mm. mercury. The pulse rate was 130 and the rate of flow 180 cc. a minute.

Autopsy shows irregular petechial hemorrhages in the muscles and fascia of the hind legs.

Slight hemolysis was noted in the 250 cc. of perfusate recovered at the end of perfusion. One hundred and sixty-one cubic centimeters of the centrifugalized end-product were injected intravenously into a normal 13.75 pound dog. A temperature rise of 0.4° was observed. No vomiting or diarrhea occurred. Twenty cubic centimeters of this perfusate were also injected intraperitoneally into a rat weighing 100 grams. There were no evidences of toxicity from the use of this perfusate.

These two experiments (experiments 5 and 7) give little information concerning the actual perfusion conditions but supply data concerning the production of a poison by the Locke's perfusion. There is slight positive evidence for a toxic reaction in experiment 5 but a negative reaction in experiment 7. In general we have no distinctly positive evidence that this destructive perfusion of body tissues will give demonstrable evidence of a toxic element in the perfusate. As stated above this may be explained by the capacity of the body cells to remove such poisons from circulating fluids.

*Perfusion below diaphragm with red corpuscles suspended in modified Locke's solution*

*Experiment 9.* Normal female black and white pup. Weight 6.75 pounds.

The oxalated blood from a normal dog was centrifugalized and the corpuscles washed in gelatin-Locke's solution minus calcium by mixture and recentrifugalization. The red cells were then suspended in gelatin-Locke's minus calcium in the ratio of packed corpuscles 1 to solution 5. This mixture was used as the perfusion medium.

Under ether anesthesia the animal was bled. Cannulae were inserted and the perfusate forced through the aorta and recovered from the right auricle, thus perfusing the area below the diaphragm. The temperature of the perfusate varied between 35° and 38°C. The mean pressure was maintained at 50 to 80 mm. mercury; the pulse pressure between 10 and 15 mm. mercury. The pulse rate was 130 a minute. The flow was excellent. Perfusion began 20 minutes after bleeding and was continued for 1 hour.

The autopsy was delayed for a few hours after the completion of the perfusion. The muscles of the hind legs were pale and showed very little edema. No hemorrhages were present in the muscles and connective tissue. The liver was normal except for some air bubbles in its vessels. The capsule of the kidneys stripped readily. Hyperemia was seen at the cortico-medullary boundary. Suprenals were negative. The lymph nodes of the mesentery were normal.

The pancreas showed a considerable amount of edema. The spleen was dark red except for a transverse light band possibly caused by block from emboli. The intestines were filled with red mucoid material. The mucosa was velvety, swollen and deep purplish-red in color. Histological sections of liver and intestine give no evidence of tissue abnormality.

On centrifugalization a sample of perfusate taken 10 minutes after beginning of perfusion was light pink. A sample taken at the end of 40 minutes was somewhat deeper in color, while a sample taken at the end of perfusion was dark red.

One hundred and fifty cubic centimeters of a centrifugalized sample taken after 10 minutes of perfusion were injected into a small normal dog. The animal vomited once. There was a rise of 1°C. in temperature. The pulse remained good. No marked symptoms of intoxication were present.

Eight cubic centimeters of the 10-minute sample were injected intraperitoneally and 2 cc. were injected subcutaneously into a 75-gram rat. No toxic action was noted. This test was repeated by injecting 5 cc. intraperitoneally into a 38-gram rat. No toxic action was noted. The same amount of the 40-minute perfusate sample was injected intraperitoneally into a 62-gram rat. No toxic action.

*Perfusion below diaphragm with red blood cells in modified Locke's solution*

*Experiment 11.* Female shepherd pup. Weight 2280 grams.

In preparation of the perfusate blood corpuscles were obtained from the blood of a normal dog bled several hours previously into a 1 per cent sodium oxalate solution. The red cells were centrifugalized and washed in Rous' gelatin-Locke's solution minus calcium. The packed cells were then suspended in a similarly prepared calcium-free gelatin-Locke's solution in the ratio of one part of corpuscles to five of the saline mixture.

Under ether anesthesia the animal was bled and the cannulae were arranged to perfuse all of the tissues below the diaphragm. Ten minutes were consumed in arranging the cannulae. The temperature of the perfusate was between 35° and 38°C. The pulse pressure was about 10 mm. mercury. Due to clots in the gauze two stops were necessitated over a period of 5 minutes each. One occurred soon after the beginning of perfusion and one some minutes later. The duration of the perfusion was 1 hour. During this period the animal increased 760 grams in weight.

Autopsy showed about 75 cc. of pale bloody fluid in the abdominal cavity. Marked edema was present about the pancreas and throughout the mesentery. Hemorrhagic spots were observed over the surface of the kidney and stomach, about the ovary and throughout the muscles and fascia of the hind legs. The liver on section was translucent. On section the kidney showed indefinite dark hemorrhagic spots up to 0.5 cm. in diameter. The whole organ was dark and congested. The stomach contents were normal. Mucus and bloody fluid were present in the small intestine. Congestion and bloody intestinal contents were more prominent in the lower part of the small intestine.

The perfusate showed a moderate grade of hemolysis before perfusion but less after 15 minutes of perfusion. Moderate hemolysis existed at the end of the perfusion.

Bacteriological cultures showed 500 bacteria per cubic centimeter in samples taken at end of perfusion.

Two hundred and eight cubic centimeters of the perfusion fluid obtained at the end of the perfusion were injected intravenously into a normal small dog. With the exception of a temperature rise of 1.5° and some shivering there were no signs of intoxication.

The two experiments (expers. 9 and 11) show the results of a perfusion of all the organs and tissues below the diaphragm by a red cell Locke's solution mixture. It is to be noted especially that there is marked edema of retroperitoneal tissues and the pancreas. This edema is invariably present in considerable amount except when whole defibrinated blood is used as perfusate. We accept the edema as one indication of tissue or cell injury. The same is true of hemorrhagic areas and ecchymoses, but some of them may be due to emboli. The marked congestion of the intestinal mucosa with the escape of blood-tinged fluid and mucus is also a valuable index of injury. This is a familiar reaction noted in dogs dead from anaphylaxis or large doses of proteose or from surgical shock. The perfusate in both these experiments contained no poisonous substance for normal dogs and white rats.

Hemolysis is always present in slight or moderate degree in all our experiments. We are inclined to explain a part of this hemolysis by the cell injury in organs or tissues and this cell injury reacts unfavorably upon the red cells with resulting hemolysis. We realize that the dog's red corpuscles are most fragile and that the red cells are subjected to much mechanical injury in these experiments. Other observers may choose to explain all this hemolysis upon a purely traumatic basis.

*Perfusion below diaphragm with diluted defibrinated blood*

*Experiment 13. Male bull pup. Weight 2150 grams.*

Two parts of defibrinated blood obtained from a normal dog were diluted with one part of gelatin-Locke's solution. The animal was anesthetized with ether, the thorax opened and the cannulae inserted in such a way that the perfusion medium was forced into the aorta just above the diaphragm and the blood received from the inferior vena cava just below the heart. In this way the entire region below the diaphragm was perfused. The temperature of the perfusion medium was maintained at about 32° to 38°C. The systolic pressure varied from 95 to 120 mm. mercury with a pulse pressure of 20 mm. mercury. The return flow was accidentally occluded for a few seconds at the beginning of the experiment. The perfusion lasted 1 hour.

Examination of the region perfused showed hemorrhagic streaks in the diaphragm and gall bladder. Numerous small areas of hemorrhage accompanied by a considerable amount of edema existed about both kidneys. The liver showed considerable congestion and edema. The lobules of the pancreas were

distinctly separated by edema. The spleen was quite dark. The stomach showed considerable engorgement with sub-mucous hemorrhages. Externally the small intestines were spotted by numerous small subserous hemorrhages. The intestinal mucosa showed diffuse congestion while the lumen contained a little dark mucus. The colon was more nearly normal in appearance; however, a few small sub-serous hemorrhages were seen. The hind legs showed no hemorrhage or edema. There was a weight increase of 400 grams.

The perfusate showed slight hemolysis before perfusion and a moderate grade of hemolysis at the end of perfusion. The perfusate obtained at the end of perfusion contained 39 mgm. of non-protein nitrogen and 16 mgm. of urea-nitrogen per 100 cc.

TABLE 34

*Perfusion below the diaphragm with defibrinated blood. Experiment 16*

TIME	COLOR OF CENTRIFUGALIZED PERFUSATE	CARBON DIOXIDE CAPACITY PER 100 CC.	HYDROGEN ION CONCENTRATION	REMARKS
Before.....	Pale pink	38.5	7.5	More blood added
After 20 minutes.....	Rose	15.7	7.3	
After 40 minutes.....	Rose	26.1		
At the end.....	Deep rose	12.8	7.4	

*Perfusion below the diaphragm with defibrinated blood*

*Experiment 16.* Normal male bull pup. Weight 4.2 pounds.

Under ether anesthesia cannulae were inserted for perfusion below the diaphragm. This region was perfused for 30 minutes with pure defibrinated blood at a rate of 100 cc. per minute. The cannulae were then shifted to the lower abdominal vessels and the hind legs perfused at a rate of 25 cc. a minute for 60 minutes, with the same perfusate. The perfusate was aerated with pure oxygen and was maintained at a temperature varying from 36° to 39°C. The pulse rate was 141 a minute.

Intestinal peristalsis was quite conspicuous at the beginning of perfusion but was less noticeable after the perfusion had been in progress for about 5 minutes. During this period the abdominal wall was very sensitive to touch and contracted violently when touched.

At autopsy a large amount of clear straw-colored fluid was noted in the abdominal cavity. The animal had gained 200 grams in weight as a result of the perfusion. The liver was slightly translucent although apparently normal. The spleen was somewhat congested. The pancreas showed no edema. The kidneys were slightly congested in the pyramidal areas. The stomach was normal. The duodenum was likewise normal but the ileum showed a mucosa congested and dark red with grey mucoid material in the lumen. The hind legs showed no edema and were quite dry in appearance. A few petechial hemorrhages appeared in the fascia.

*Histological sections:* Pancreas and kidney are normal. The spleen and liver show capillary congestion but normal parenchyma cells. The stomach and small intestine are normal except for slight congestion of the ileum.

In these two experiments (experiments 13 and 16) we used diluted or whole defibrinated blood. The general autopsy picture following the use of whole blood is almost normal and the lack of the edema we believe is to be explained on this ground. Even in the last experiment we note the development of ascites which of course indicates circulatory abnormality. There is further a distinct acidosis to be explained by inadequate aeration of the blood.

*Perfusion with whole defibrinated blood—toxic proteose added*

*Experiment 17. Adult male poodle dog. Weight 10.25 pounds.*

Under ether anesthesia the animal was bled. The arterial cannula was placed just above the bifurcation of the iliacs. The venous cannula was inserted in the inferior vena cava just above the renal veins. Thirty minutes were consumed in bleeding, arranging the cannula and starting the perfusion flow. The hind legs were perfused for 32 minutes with defibrinated blood. To the 500 cc. of perfusate then remaining in the apparatus 100 cc. of a *proteose solution* (lethal dose is 2 cc. per pound body weight, adult dog) were added and the perfusion continued for 12 minutes. The perfusate was warmed to a temperature of 35° to 38°C. The rate of flow was 80 cc. per minute until near the end of the experiment, when it decreased to 50 cc. per minute. The aeration of the perfusate was excellent. The proteose solution was prepared as described elsewhere (23) from the material of the obstructed intestine.

Autopsy of the perfused dog showed slight icterus of the tissues of the hind legs but no hemorrhages or edema. A few small hemorrhages were found in the right testicle. The pelvic organs were negative.

At the end of perfusion there were obtained 300 cc. of perfusate for analysis. Of this were injected into a 14-pound normal pup, 227 cc., which represented 38 cc. of the original proteose. This would be a fatal dose for an adult dog weighing 19 pounds, provided no proteose had been lost. Pups are more susceptible to proteose intoxication than adults. This dog therefore received a theoretical dose of one and one-half times its lethal dose, assuming that no proteose was lost from the perfusate during the perfusion.

The injection of 227 cc. of perfusate caused death in 3 hours with the clinical picture of acute proteose intoxication. Diarrhea and vomiting appeared within  $\frac{1}{2}$  hour after injection and continued until death. There was an initial rise in temperature followed by a drop to 36.8°C. half an hour before death. Autopsy findings showed exquisite splanchnic engorgement especially marked in the mucosa of the small intestines, which was a velvety purplish-red coated with mucus—described and pictured elsewhere (24).

The results of examination of the perfusate are given in the table below (table 35).

*Perfusion with whole defibrinated blood—toxic proteose added*

*Experiment 18.* Under ether anesthesia a small female mongrel dog was bled. Cannulae were inserted preparatory to perfusing all of the body below the diaphragm. Defibrinated blood warmed to 35° was perfused through this region under a mean pressure of 110 mm. of mercury. The pulse pressure varied between 5 and 15 mm. of mercury. The pulse rate was 120 per minute. Aeration of the perfusate was not quite as satisfactory as usual. Actual perfusion commenced 30 minutes after bleeding the animal. After the flow has been maintained for 20 minutes 100 cc. of proteose solution were added to the 250 cc. of defibrinated blood then remaining in the apparatus. Perfusion was continued for 15 minutes. The rate of flow was 150 cc. a minute at first, but gradually decreased to 85 cc. a minute toward the end of the experiment. Marked intestinal peristalsis was present during the first few minutes of perfusion; blood-tinged feces later.

Autopsy showed a moderate quantity of pale blood-tinged fluid in the peritoneal cavity. The animal had gained 180 grams in weight during the experiment. The liver showed edema and small hemorrhages throughout. Numerous small hemorrhages as well as several larger ones were seen in the wall of the gall bladder. The pancreas was negative except for one small hemorrhage. The mesenteric lymphatics contained blood-stained fluid. The adrenals contained several small hemorrhagic areas. The kidneys were negative. The spleen was small, dark and translucent. The stomach showed one fairly large but no small hemorrhages. The mucosa of the duodenum was engorged. The intestinal lumen contained an excess of thin bloody fluid with little mucus. It is possible that some of the hemorrhages noted above are to be explained by short periods of high blood pressure during the periods of perfusion.

As was stated above, 100 cc. of the proteose solution (lethal dose is 2 cc. per pound body weight, adult dog) were added to the 250 cc. of perfusate then in circulation and the perfusion continued for 15 minutes. Of the final perfusate 77 cc. were injected intravenously into a normal 11-pound pup (no. 17-181). It is evident that these 77 cc. contained 22 cc. of the original proteose solution provided none of this toxic material had been removed during perfusion by the tissues of the animal perfused. The reaction of the animal might be expected therefore to be lethal, if no proteose was removed during the perfusion through the tissues of the first dog. The reaction to this intravenous injection was typical for a *sublethal* toxic dose of proteose. There was vomiting and diarrhea for 2 hours and much prostration. Recovery was evident in 3 hours and the dog was normal in a few more hours. It appears that some of the proteose had been removed as the amount given was more than a lethal dose for a pup of 11 pounds body weight.

The final perfusate was further examined and shown to contain definite amounts of hemoglobin (hemolysis). Bacteriological examination (plates) showed 40,000 colonies per cubic centimeter of the perfusate. The non-protein nitrogen at beginning of perfusion was 34.7 mgm. per 100 cc. of perfusate and at the end was 35.8 mgm.

In these two experiments (experiments 17 and 18) the perfusion was done with whole defibrinated blood to insure a minimum injury of the perfused tissues. After the initial perfusion a standardized toxic solution was added to the perfusate and again circulated as before. The perfusate at the end of the experiment was tested on a normal dog and in this way it was demonstrated that some of the poison had been removed. It is easy to show that large doses of this toxic proteose are rapidly removed from the circulation of a dog. Following intravenous injection of large amounts of toxic proteose it is possible to demonstrate its presence for 2 to 3 minutes in the blood stream but not after 5 minutes. The presence of bacteria as noted in this and other experiments will not seriously disturb the reaction. If anything, their presence will increase the toxicity of the perfusate mixture. These bacteria probably gain

TABLE 35

*Perfusion with whole defibrinated blood—toxic proteose added. Experiment 17*

TIME	HEMOLYSIS	PER CENT BLOOD CELLS	UREA NITROGEN	NON- PROTEIN NITROGEN	HYDROGEN ION	CARBON DIOXIDE CAPACITY
			mgm. per 100 cc.	mgm. per 100 cc.		
Before perfusion.....	Moderate	47	19	26	7.5	16
After one-half hour....	Moderate	50	16	31	7.4	16
On adding proteose....	Moderate	47	18	30	7.4	19
At end of perfusion....	Slight	45	20	89	7.4	13

entrance in part through the intestinal tract and in part are added by the manipulation of the perfusate. Efforts were made to preserve the circulating machinery in as near a sterile condition as possible but there are many possibilities for introducing contamination.

The "proteose solution" used in these experiments is prepared from material obtained from obstructed intestines or closed intestinal loops in dogs. The material is precipitated by five volumes of alcohol, the precipitate dissolved in water and the protein removed by boiling in dilute acetic acid solution. A second precipitation with alcohol is often employed. The final solution is an opalescent fluid which contains proteose-like materials. This fluid material is then standardized by intravenous injection in normal dogs, as has been described elsewhere (23).



*Large infusion of Locke's solution into portal vein*

*Experiment 21.* Dog 17-125. Normal adult mongrel, black and tan. Weight 43 pounds.

Under ether anesthesia and with sterile precautions the abdomen was opened and the hepatic-pancreatico-duodenal and pancreatico-duodenal arteries were ligated. Sterilized calcium-free Locke's solution, 1750 cc., was warmed to approximately 36°C. and injected at a rate of 55 cc. a minute into the portal system through a small venous branch in the mesentery. The abdomen was closed. The animal showed no severe reaction for some time but died 3 days later.

Autopsy performed several hours later shows considerable post-mortem change. Dark red softened areas containing bubbles of gas are seen scattered throughout the liver. The serosa of the intestines is quite red. The mucosa shows moderate engorgement and is covered by a buttery exudate. The coil of intestine to which the perfused vein was distributed differs in no way from the other parts of the small intestine. Kidneys are negative. The total non-protein nitrogen of the blood shows but slight alteration from the original value as a result of the experimental procedure.

*Histological sections:* The picture is somewhat confused by post-mortem changes but it is clear that there are scattered areas of liver cell necrosis which are ante-mortem and presumably related to the experimental procedure. These areas include many liver lobules and present a uniform necrosis with scattered leucocytes between the liver cell strands. Bile ducts and blood vessels are normal and no evidence of vascular thrombosis is observed in any sections. Other sections show normal liver parenchyma. Other tissues present nothing of importance.

*Large infusion of Locke's solution into splenic artery*

*Experiment 23.* Dog 17-200. Young male collie. Weight 29.5 pounds. Normal except for a slight attack of distemper.

Under ether anesthesia and with sterile precautions the abdomen was opened. A cannula was inserted into the splenic artery in such a manner that the upper arm of the pancreas, a part of the duodenum and stomach, as well as the liver, were perfused by 2000 cc. of sterile calcium-free Locke's solution injected at a rate of 50 cc. a minute. The saline was injected at room temperature. Splenectomy followed the perfusion. The pancreas showed moderate edema at the end of the experiment, but the dog was not severely shocked. Later a state of intoxication slowly developed and at the end of 36 hours death was imminent. The animal was killed by ether.

Autopsy performed at once shows a little blood-stained peritoneal fluid. The liver shows a moderate grade of cloudy swelling. The subserous tissues of the gall bladder are thick and edematous. A number of sub-serous ecchymoses are scattered over the small intestine. Several hemorrhagic areas are present in the mucosa of the small intestine. Hemorrhages and fat necrosis are rather pronounced in the upper arm of the pancreas.

*Histological selections:* Organs are normal with the exception of the pancreas and liver. The pancreas shows extensive hemorrhagic necrosis and much nec-

rosis of fat and gland parenchyma. There are no thrombus masses noted in any of the vessels. The head of the pancreas is essentially normal. The liver shows scattered clumps of polymorphonuclear leucocytes and evidence of injury to small clusters of liver cells in various portions of the liver lobules.

*Large infusion of Locke's solution into the portal vein*

*Experiment 24.* Dog 17-203. Adult male mongrel. Weight 29.5 pounds. Slight distemper.

Under ether anesthesia the abdomen was opened and the hepatic artery clamped. One of the larger splenic veins was isolated, a cannula inserted and 1500 cc. of sterile calcium-free Locke's solution warmed approximately to body temperature were injected into the portal system over a period of 18 minutes. The clamps were then removed from the hepatic artery. Splenectomy was performed and the abdomen was closed. The temperature rose to 40°C. shortly after the operation and the animal vomited once; otherwise no clinical disturbance was noted. Complete recovery ensued. The animal was killed 7 days later. The autopsy was negative. No alteration could be made out in the liver.

To obviate the mechanical difficulties inherent in organ perfusion and to get information concerning the direct effect of Locke's solution upon tissue and organ cells we performed a number of experiments of which experiments 21, 23 and 24 are examples. The first experiment (exper. 21) shows a fatal reaction following a large infusion in the portal vein after ligation of the branches of the hepatic artery to limit the blood flow through the liver. We have explained this reaction as due in part to injury of the liver cells by contact with the mixtures of Locke's solution and blood. It is known that ligation of the hepatic artery will cause no disturbance in the dog. It seems hard to account for these areas of liver necrosis except as due to the action of the portal blood diluted by the large infusion of Locke's solution into the portal vein. It is to be noted (exper. 24) that a similar experiment was tolerated without obvious liver injury but the occlusion of the hepatic artery in this experiment was only temporary.

The pancreas necrosis was surely caused by the perfusion of the splenic artery (exper. 23). It may be objected that perfusion in this way against the arterial stream will cut off the tissues from oxygen by washing away all available red cells. We are inclined to believe that arterial collaterals which are numerous in this region will insure the presence of the necessary number of oxygen-carrying red cells. This objection cannot apply to experiment 21.

## DISCUSSION

In attempting an analysis of our own experiments we wish to draw freely on the published work of other investigators. We wish to keep constantly in the reader's mind that *physiological perfusion* of any organ is a matter of extreme difficulty and often great confusion is introduced by such methods which are intended to simplify the study of organ function. By *physiological perfusion* we mean a perfusion adequate to maintain the organ in its normal physiological activity.

In the first place let us inquire what criteria of tissue abnormality we have. What sort of evidence is going to lead us to pass judgment concerning the physiological condition of tissues? There are certain conditions under which we may ascertain what is going on in the tissues by a direct observation of the functional activity of the part perfused. For example, in perfusing the kidney the quantity and quality of the urine secreted furnishes some evidence concerning the condition of that organ. The reduction within the organ of oxyhemoglobin to hemoglobin was noted by the earliest observers and is indicative of metabolic activity of some nature on the part of the perfused tissues. The nature of the heart beat is indicative of the condition of the perfused heart, although Magnus (25) has shown that if such an inert substance as hydrogen gas be perfused through the coronary arteries heart beats will be stimulated. Sollmann (26) showed that the same result followed perfusion with cottonseed or paraffin oil. In such cases the perfusion fluid cannot be thought of as being a nutrient fluid; on the other hand it is not altogether impossible that, as was suggested by Sollmann, the heart beats may be stimulated by purely mechanical factors. For these reasons we must not hastily conclude that, because the gross mechanical movements simulate those occurring in the intact animal, the preparation is in fact an example of normal physiological activity.

A criterion as to the condition of the perfused medulla is furnished by observing whether the medulla continues to maintain its normal control over the heart and muscles of respiration.

In addition to direct observation of the functional activity of an organ perfused, we have still other kinds of evidence which help us to judge concerning the condition of the tissue. Thus the rate of flow through the vessels is in some cases a valuable indicator for it is a general rule that tissue injury brings about in some way or other a decrease in the rate of flow through the part. We have, in addition, the still more crude signs of tissue injury: edema, congestion and hemorrhage.

Of the factors in the procedure of perfusion whose variations might bring about injury to the part, the following may be mentioned as being perhaps the most important: aeration of the perfusion medium, composition of the perfusion medium, interruptions in continuity of flow, temperature of the perfusion medium when it enters the perfused organ, mean pressure and pulse pressure.

It should be realized that a perfusion experiment is no better than its weakest point. If any of the above factors react in such a way as to cause injury to an organ, perfection of the other factors will not remedy the defect. It is also conceivable that when several organs are being perfused simultaneously, injury to one organ or tissue may react injuriously on others.

Concerning the effect on the tissues of composition of the perfusate, the literature contains many references to condition of the tissues as shown by functional activity. The injurious effects of foreign blood have been known since the time of Prévost and Dumas (27) when this fact first began to be recognized through the failure of foreign blood to act normally after transfusion. Though repeatedly shown to be harmful in its effects on tissues of another species, foreign blood has been used in perfusions even as late as Brodie (13) who says that ox, sheep or horse blood cannot be used in the perfusion of organs taken from dogs. He finds that as soon as foreign blood is supplied to the perfused heart the beat becomes irregular. The heart next goes into fibrillary twitchings and cannot be recovered from this state even with the animal's own blood.

Although defibrinated blood had been used in the transfusion experiments of Prévost and Dumas (27) without the observation of harmful results, Magendie found it incapable of carrying on the normal function of the circulating medium after transfusion. In a series of experiments in 1822 (28) and again in 1838 (29) he presented experimental data to show that the lack of fibrin, reduced through repeated bleeding, defibrination and reinjection of the defibrinated blood gives rise to a serous and bloody transudate into the lungs and intestine with the death of the animal.

The weight of Magendie's name behind such a statement did much to discredit defibrination in the eyes of other workers, but eventually Bischoff (30), Goll (31), Polli (32), Panum (33), Ponfick (34), and many others began to turn the weight of experimental evidence against a belief in the extreme toxicity of defibrinated blood when used in transfusions between animals of the same species

More recently, by means of perfusion experiments, Stevens and Lee (35) and Brodie (13) present evidence of slight vasoconstriction due to the use of defibrinated blood as a perfusion medium. Their work again points to an injurious action of defibrinated blood when substituted for the normal circulating medium. However, Stevens and Lee believe that the slight vascular contraction which they note can be readily counteracted with pharmacological agents.

In 1903 Pfaff and Vejux-Tyrode (36) found defibrinated blood definitely injurious to the kidney of the dog. Quantities of from  $\frac{1}{4}$  to  $\frac{1}{10}$  of the total blood were withdrawn from the carotid artery, whipped, filtered and reinjected into the jugular vein. The repetition of this procedure resulted in the appearance of albumin, hemoglobin and red blood cells in the urine and finally cessation of secretion. However, a rapid return to normal was effected in these animals by bleeding followed by direct transfusion of whole blood from a normal dog. It would seem that the kidney may be unusually sensitive to this procedure.

It is certain that in those of our animals which underwent quite complete defibrination (37)—(see expts. 323 and 324)—there were no clinically evidenced signs of injury or toxic manifestations. This may also be said of those experiments of Whipple and Goodpasture (38) in which quite complete defibrination was also effected.

The importance of a physiological balance of the normal inorganic salts of the blood is generally recognized. Solutions containing abnormal quantities of these salts have been shown to be toxic to the perfused heart. Hooker (22), (39) showed that in perfusion of the respiratory center a balance of potassium and calcium is essential for a normal function of the preparation. As was early shown by several investigators and more recently reemphasized by Hooker (19) and by others, and as we have found in our experiments, saline solution has the property of setting up such a condition in the tissues that the rate of flow gradually decreases.

The effects of variations in composition of perfusion media may also be manifest from the morphological side. Brodie (13) shows that edema results from the use of foreign blood. Hamel (10) shows that edema results in organs perfused with saline under pulsatile pressure. Similarly the kidney when perfused with Locke's solution exhibits more edema then when perfused with defibrinated blood (19). These results are entirely in accord with our experience. Perfusion with pure Locke's solution almost invariably produces an extreme grade of edema. Dog's defibrinated blood diluted with Locke's solution produced less,

and pure defibrinated blood produces very little demonstrable edema. It is difficult to explain just why dilution with Locke's solution should produce edema. In several of our experiments we carried out hydrogen-ion and carbon dioxide determinations on the perfusate and it is interesting to note that in cases of marked edema there was a rise in the hydrogen ion concentration and a fall in the carbon dioxide capacity. Whether the edema is the result of the acidosis or not, there still remains the question as to what is the cause of the decrease in the buffer substances. Perhaps it may be attributable in part at least to insufficient oxidation in the tissues. Poor oxidation may result from insufficient oxygen-carrying capacity of the perfusate or from inadequate aeration of the perfusate in the artificial lung, or may result from a decreased rate of flow through the animal and a stagnation of the blood in the tissues with consequent asphyxia.

A factor which has been shown to be of considerable importance in causing tissue injury is that of loss of time in establishing the artificial perfusion after interrupting the normal relations. Most of the earlier workers paid but scant attention to this phase of the problem. In many experiments several hours elapsed before any attempt was made to reestablish the flow. Grube (40) mentions that in perfusion of the liver with defibrinated blood to which glucose had been added, the glycogen content of the liver rises, but only in case the circulation is very promptly reestablished. The deleterious effect on the kidney of temporary anemia is well known. Momentary compression of the renal vessels may cause a cessation of secretion for many minutes. The effect of compression of the cerebral vessels has been known since very early times. The duration of such anemia necessary to produce irreparable damage was long ago studied by Astley Cooper (41). Signs of activity can be restored to the brain of the isolated head provided only that the perfusion is promptly commenced (6), (42), (43) and (44). Skeletal muscle is capable of surviving much longer periods of anemia than is the case of brain or kidney. Munk (45) holds that in perfusion of the kidney if the flow is not promptly commenced the vessels become narrowed, the perfusion flow rendered difficult and a delay in the formation and flow of urinary fluid occurs. Recently Bainbridge and Evans (21) have succeeded in perfusing the kidney without any interruption whatsoever in the continuity of flow.

A great deal has been said in the literature concerning the value of pulsatile pressure. As has already been noted, Ludwig and Schmidt (7) observed that with constant pressure the rate of outflow from the

perfused tissue decreases, but that recovery occurs if the flow is halted for a while. Similar observations were subsequently made by many workers. The recognition of the importance of this factor is evidenced by the numerous forms of apparatus devised to accomplish this end. Hooker (19) holds that in the perfused kidney the amount of urinary filtrate formed varies directly with the magnitude of the pulse pressure. The amount of proteins in the urinary filtrate varies inversely with the magnitude of the pulse pressure. The rate of blood flow through the organ varies directly with the magnitude of the pulse pressure. Recently Gesell (46) has been able in the intact animal to abolish almost completely the pulse pressure in the renal arteries without interfering with the normal mean blood pressure. The result of this alteration was an immediate and practically complete cessation of urinary secretion.

#### SUMMARY

Physiological perfusion of organs is a matter of great difficulty. Much of the work done with organ perfusion is of little value because a proper appreciation of the limitations of the method does not obtain among laboratory workers.

The use of physiological saline, Locke's solution or various modified solutions with or without red blood corpuscles does not permit of *physiological perfusion* of organs. The contact of these salt solutions with the tissue cells will result in profound injury or actual cell destruction. Any deduction made from experiments of this nature must be limited by these facts just outlined.

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# THE SEASONAL VARIATION IN THE GROWTH OF BOSTON SCHOOL CHILDREN

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## I

In 1892 I secured the measurement of weight and height, and other physical dimensions, from 34,500 boys and girls in the public schools of St. Louis. In this investigation, as in similar studies upon which our present standards are chiefly based, all the measurements were collected at one time, once for all. The children were then distributed by sex and age, and the median weight and height, etc., determined for each year. This is the "generalizing" method. Its economies are great. Thus, in the St. Louis investigation, a little more than a million data were harvested in eleven weeks, and work in the class room was interrupted for only seven half-hour periods.

Far different is the "individualizing" method. This procedure demands the measurement of the same child again and again, throughout its period of growth. The measurements must begin at the earliest age with many thousand children, lest death and desertion so thin the ranks that the survivors will be too few for safe statistical treatment. The individualizing method demands, therefore, a formidable expenditure in time and effort through many years. Toilsome as this task may be, it cannot be foregone. For the generalizing method conceals a grave flaw; it does not give the growth of the individual child.<sup>1</sup>

With this in mind, I asked the Boston School Committee, in 1909, to measure the height and weight of several thousand of the youngest children and to repeat the measurements monthly in the same children throughout their school life. The measurements were made by the school nurses, under the direction of Dr. T. F. Harrington, and after his lamented death they were continued by his successor, Dr. W. H.

<sup>1</sup> This defect in the generalizing method and the contrasting advantages of the individualizing method were discussed by me in the Quarterly Publications of the American Statistical Association, December, 1893.

Devine. In this land of criticism, in which our minds are often occupied with the real, and still more often with the imaginary defects of our public institutions, the patient, laborious collection of these measurements, month after month, year after year, is a monument of devotion in which we may all take pride.

## II

These measurements of the weights and heights of Boston children throughout their school life were completed in June, 1919. The statistical analysis began the following month. The first step was to distribute the weights according to age in months. For example, all boys 110 months of age were placed in one group, and the median weight for that group was calculated. It will be observed that the principle of this first distribution is still that of the generalizing method. This principle must be clearly apprehended. In the generalizing method the individual measurements are distributed into groups, by which distribution the personal character of each individual is lost—the individual becomes merely a statistical unit. Whether the measurements so distributed have been made monthly, or yearly, or whether all the children are measured but once and the measurements distributed by years of age, is immaterial; the essential mark of the generalizing method is the loss of personal identity—the transformation of the boy or girl into a statistical unit.

In this present investigation the first distribution of boys' weights, described above, is therefore a distribution by the generalizing method. Its fruits are given in table 1, which records the median weight of boys at each month of age from the 60th to the 176th month, inclusive. In this table, the months are not calendar months, but months of age. For example, the weight opposite 110 months is the median weight of 1226 boys born in 1904, 1905 and 1906. Of these, 33 were born in October, 1904; 71 in January, 1905; and 10 in April, 1906. The October boys reached 110 months of age in December, 1913; the January boys in March, 1914; and the April boys in June, 1915.

When the data in table 1 are plotted, there results the curve shown in figure 1. This curve of monthly growth is in principle that also obtained when the median value is calculated for each year, and these annual values are connected by a line—the curve of growth, old style. These curves rise steadily and smoothly in an unbroken line.

Such curves have given rise to much loose thought; for a curve obtained by the generalizing method is a statistical and not a personal

TABLE 1

*Weights at each month of age from 60 through 176. Boys born in 1904, 1905 and 1906*

MONTHS	POUNDS	MONTHS	POUNDS	MONTHS	POUNDS	MONTHS	POUNDS	MONTHS	POUNDS
60	42.75	72	44.00	84	47.68	96	52.48	108	57.74
1	41.10	3	44.25	85	48.30	7	52.96	9	58.34
2	41.30	4	44.35	6	48.78	8	53.74	110	58.64
3	42.00	75	45.22	7	49.29	9	53.90	1	59.51
4	41.44	6	44.97	8	49.80	100	54.41	2	60.01
65	42.26	7	45.65	9	50.09	1	54.91	3	60.54
6	42.31	78	45.67	90	50.56	2	55.27	4	60.94
7	42.06	9	46.09	1	50.73	3	55.87	115	61.58
8	41.98	80	46.34	2	51.25	4	55.83	6	61.56
9	42.56	1	47.03	3	51.32	105	56.27	7	61.83
70	43.17	2	47.18	4	51.85	6	56.76	8	62.36
1	44.53	3	47.47	95	52.22	7	57.23	9	62.95
120	63.42	132	69.42	144	75.21	156	82.19	168	92.75
1	64.22	3	70.17	145	76.23	7	83.56	9	93.13
2	64.96	4	70.90	6	76.44	8	84.43	170	93.88
3	65.14	135	71.60	7	77.48	9	85.94	1	97.00
4	65.69	6	72.85	8	78.17	160	86.66	2	92.67
125	66.46	7	72.92	9	78.55	1	87.56	3	94.00
6	66.97	8	73.13	150	79.82	2	88.47	4	96.50
7	67.49	9	73.67	1	80.24	3	89.44	175	96.00
8	67.67	140	73.72	2	80.22	4	90.17	6	95.50
9	68.31	1	74.50	3	80.96	165	91.58		
130	68.49	2	74.43	4	80.98	6	92.25		
1	68.97	3	74.90	155	81.53	7	91.19		

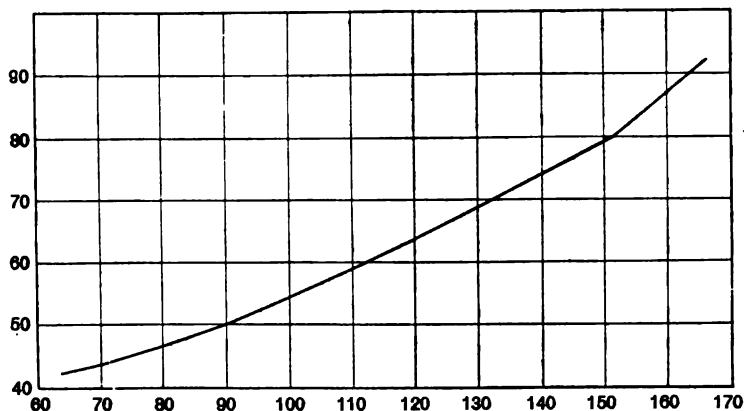


Fig. 1. The median weight of boys at each month of age from the 60th to the 176th month, inclusive (table 1). In this figure the months are not calendar months but months of age. The ordinates are pounds.

phenomenon. This will be clear, if we consider the personal history of two boys, whom we will call John and James. At age six, John is 30 per cent above, and James is 30 per cent below the average. But John's family fall upon evil days; illness and poverty pursue them. The family of his comrade prosper; James spends his vacations in the country, where he has a horse to ride; fresh air and good food do their accustomed work. At age sixteen, James and John have exchanged places; James has risen to 30 per cent above the average and John has fallen to 30 per cent below it. Their personal fortunes have altered; but they are still the same distance from the average. The inhuman average is unmoved by the deplorable fate of John and the happy success of James—to the statistical average, John and James are not persons, but statistical units. Thus the generalizing method *per se* gives no accurate information as to the growth of the individual child. Nor can this defect be removed by measuring repeatedly the same children throughout their period of growth, either monthly or at any other interval, so long as the resulting measurements are treated as statistical units. Thus, table 1 and figure 1 give accurately the monthly increase in the statistical median value, and they give with accuracy the relation between the size of any individual child and the size of other children of the same age; but they do not give, with certainty, the increase in weight of any child. They are not standards of growth, but merely standards of relative size.

### III

The extinction of the individual is not the only indictment which can be brought against the generalizing method. This method, still so much employed, is blind to the possibility of seasonal growth.

Consider the case of Hyman Katz, drawn at random from the mass of children whose growth histories are now before us. Hyman Katz was born in August, 1905. In his ninth year, he gained four pounds between September and January, whereas he did not gain any weight, to speak of, between February and June. The case of Hyman Katz is the starting point of a series of interesting and important observations. It will be seen that Hyman Katz is not an exception, save that he is a somewhat extreme illustration of a law binding on other boys and girls.

Examine table 3 which deals with all boys and girls born in August, 1905. Again the growth in weight is much larger in the second half

TABLE 2

*Growth in pounds of Hyman Katz 1914-15*

MONTH	WEIGHT	GAIN IN WEIGHT	MONTH	WEIGHT	GAIN IN WEIGHT
1914	pounds	pounds	1915	pounds	pounds
September	56.50		January	60.50	0.37
October	57.00	0.50	February	60.50	0
November	58.25	1.25	March	60.00	-0.50
December	60.13	1.88	April	60.00	0
			May	60.50	0.50
			June	61.00	0.50

TABLE 3

*Gain in weight of boys and girls born in August, 1905*

YEAR	BOYS		GIRLS	
	September to January	February to June	September to January	February to June
	pounds	pounds	pounds	pounds
1912-13	3.34	+0.75	1.88	0.12
1913-14	2.17	-0.17	2.50	0.65
1914-15	2.85	+0.67	2.96	0.13
1915-16	1.29	+0.81	1.63	0.71
1917-18	5.85	-0.07	3.19	0.81
1918-19	4.88	+2.90	4.63	1.83
Average.....	3.40	0.82	2.79	0.71
Ratio.....	$\frac{3.40}{0.82}$	$= \frac{4.1}{1}$	$\frac{2.79}{0.71}$	$= \frac{3.9}{1}$

The year 1916-17 is omitted because the schools were closed in September, 1916, on account of an epidemic.

of the year than in the first half. This conclusion is supported by the examination of the weights of boys born in all the months of 1905, when these weights are distributed by the months of the year. In table 4 the median weight of all boys born in 1905 is given for each month of the year, and in table 5, the increase in the median weight is recorded. Seasonal growth is again demonstrated. The data in table 4 are reproduced in figure 2.

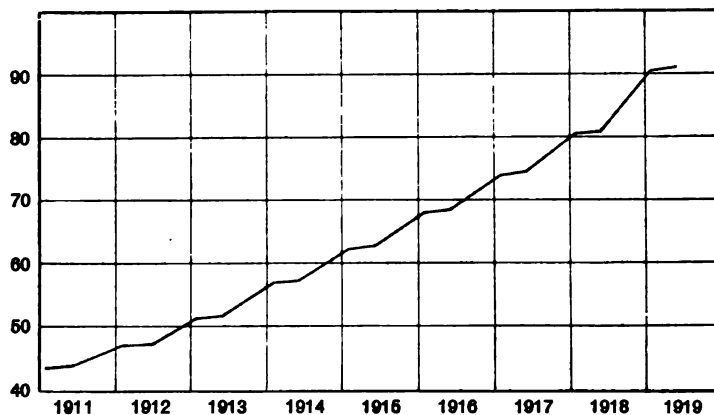


Fig. 2. The weights of boys born in 1905, distributed by months of the year. The ordinates are pounds. (See table 4.) Fig. 2 is the true curve of growth in weight; it shows the seasonal variations. Compare this figure with Fig. 1, the curve of growth "old style," in which the seasonal variations are lost.

We have dealt thus far with the absolute increase in weight. It is desirable to examine also the percentile increase, i.e., the absolute increase of each month divided by the weight at the beginning of the month. As would be expected, the seasonal variation again appears. The average total percentile gain in the first five months of the years 1913, 1914, 1917 and 1918 is 1.89; whereas in the last five months of those years, the average total percentile gain is 6.61.

TABLE 4

*The weights of boys born in 1905, distributed by months of the year*

YEAR	JANUARY	FEBRUARY	MARCH	APRIL	MAY	JUNE	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER
	pounds	pounds	pounds	pounds	pounds	pounds	pounds	pounds	pounds	pounds
1911	43.00	42.23	42.93	42.83	42.48	42.46	46.20	45.04	45.21	45.31
1912	46.82	46.70	46.62	47.03	46.99	47.03	48.98	49.22	49.98	50.38
1913	50.94	51.49	51.73	52.18	52.05	51.97	53.31	54.16	55.00	55.37
1914	56.45	56.73	57.19	57.38	56.99	56.82	58.34	59.38	60.05	60.79
1915	61.50	62.08	62.38	62.48	62.50	62.72	64.94	65.02	65.66	66.47
1916	67.09	67.88	68.34	68.58	68.32	68.53		71.50	72.18	72.89
1917	73.39	73.81	74.21	74.43	74.81	74.79	76.49	77.32	78.27	79.23
1918	80.17	79.88	81.13	81.38	80.85	81.06	84.29	87.00	87.13	88.00
1919	89.93	90.27	91.17	92.05	91.83	91.55				

In table 4, the median weight of all boys born in 1905 is given for each month of the year without regard to the month of age. The seasonal growth is thereby shown. Thus, in the five months from January to June, 1914, the gain was less than  $\frac{1}{2}$  pound, but in the five months from June to November, 1914, the gain was  $3\frac{1}{2}$  pounds.

TABLE 5

*The monthly increase in weight of boys born in 1905*

YEAR	JANUARY TO FEBRUARY	FEBRUARY TO MARCH	MARCH TO APRIL	APRIL TO MAY	MAY TO JUNE	JUNE TO SEPTEMBER	SEPTEMBER TO OCTOBER	OCTOBER TO NOVEMBER	NOVEMBER TO DECEMBER	DECEMBER TO JANUARY
1911	-0.77	+0.70	-0.10	-0.35	-0.02	+3.74		+0.17	+0.10	+1.51
1912	-0.12	-0.08	+0.41	-0.04	+0.04	+1.95	+0.24	+0.76	+0.40	+0.56
1913	+0.55	+0.24	+0.45	-0.13	-0.08	+1.34	+0.85	+0.84	+0.37	+1.08
1914	+0.28	+0.46	+0.19	-0.39	-0.17	+1.42	+1.04	+0.67	+0.74	+0.71
1915	+0.58	+0.30	+0.10	+0.02	+0.22	+2.22	+0.08	+0.64	+0.81	+0.62
1916	+0.79	+0.46	+0.24	-0.26	+0.21			+0.68	+0.71	+0.50
1917	+0.42	+0.40	+0.22	+0.38	-0.02	+1.70	+0.83	+0.95	+0.96	+0.94
1918	-0.29	+1.25	+0.25	-0.53	+0.21	+3.23	+2.71	+0.13	+0.87	+1.93
Average ...	+0.18	+0.47	+0.22	-0.16	+0.05	+2.23	+0.96	+0.61	+0.63	+0.98

For convenience we may divide the total of 2.23 pounds from June to September into 0.74 pound for each of the three months.

The errors caused by neglecting the seasonal growth are strikingly shown by comparing a series of weights chosen from table 1 and table 4. Let us take from table 1, twelve weights, beginning with 56.76 pounds, and from table 4, twelve weights, beginning with 56.73 pounds.

FROM TABLE 1—BOYS' WEIGHTS DISTRIBUTED BY MONTHS OF AGE	FROM TABLE 4—BOYS' WEIGHTS DISTRIBUTED BY MONTHS OF YEAR
<i>pounds</i>	<i>pounds</i>
56.76	56.73
57.23	57.19
57.74	57.38
58.34	56.99
58.64	56.82
59.51	*
60.01	*
60.54	58.34
60.94	59.38
61.58	60.05
61.56	60.79
61.83	61.50

\* July and August; the vacation months.

In these two series, the beginning and the end weights are almost the same, but in the middle of the twelve months the divergence is 2 pounds or more.

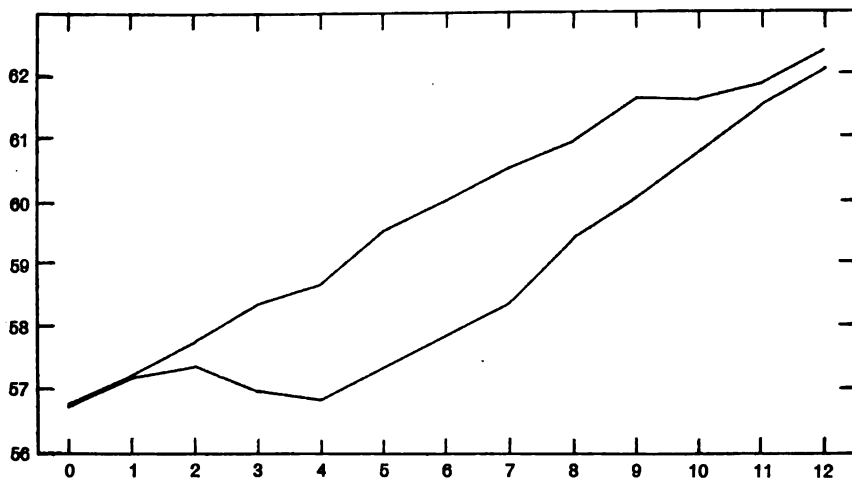


Fig. 3. The ordinates are pounds and the abscissae are months. The lower curve records boys' weights distributed by months of the year (table 4); the upper curve records boys' weights distributed by months of age (table 1).



In figure 3, the two series are shown graphically.

The enquirer will wish to know how the marked seasonal variation in weight is so completely masked in table 1 and figure 1, and in the upper curve of figure 3, examples of the statistical method hitherto in use. The answer is that in the method hitherto in use—in which the only criterion is the month of age—the month of age for half the boys will fall in a season of rapid growth, while for the other half the same month of age will fall in a season of slow growth. Compare during the months of age from 106 to 118 the growth in weight of boys born in August, 1905, with that of boys born in February, 1905 (table 6).

TABLE 6

AGE IN MONTHS	SEASON	WEIGHT	AGE IN MONTHS	SEASON	WEIGHT
<i>Boys born in August, 1905</i>					
112	December, 1914	<i>pounds</i> 59.96	118	June, 1915	<i>pounds</i> 61.56
106	June, 1914	55.25	112	December, 1914	59.96
		4.71			1.60
<i>Boys born in February, 1905</i>					
112	June, 1914	55.75	118	December, 1914	61.17
106	December, 1913	54.50	112	June, 1914	55.75
		1.25			5.42

It is clear that if the age in months is alone considered, the period of rapid growth in boys born in February will coincide with the period of slower growth in boys born in August. The two periods will compensate each other if the February and the August boys are treated *en masse*, as in table 1 and figure 1, and in the standards hitherto so largely used to determine the growth of school children.<sup>2</sup>

## IV

Rather than accept a dictum so far-reaching, the reader may here suggest that my entire contention rests on a palpable error—a failure to take into account the heavier garments worn in winter. Table 5

<sup>2</sup> My data do not yet show any seasonal variation in the height of school children.

shows a net increase of 0.76 pounds in the first five months of the year (+0.18, 0.47, 0.22, 0.05, -0.16), whereas the increase in the last five months of the year is 3.92 pounds (+0.74, 0.96, 0.61, 0.63, 0.98). But it will be urged that in May, which falls in the first five months, the winter clothing is laid aside; and in October, which falls in the last five months, it is again put on.

A careful examination of the data will show that the seasonal differences in growth cannot be explained by seasonal differences in the weight of garments worn. Consider the following groups, taken from table 5.

INCREASE FROM	INCREASE FROM	INCREASE FROM
pounds	pounds	pounds
January-February 0.18	June-September. 2.23	October-November..... 0.61
February-March. 0.47		November-December... 0.63
March-April..... 0.22		December-January..... 0.98
Average..... 0.29	0.74	0.74

Obviously, the second group is composed of summer months and the third group of winter months. The first group requires, perhaps, a word of explanation. In Boston the month of April offers the promise but not the reality of spring. The average temperature rises from 40° on April 1 to 51° on April 30. In 1919, the maximum temperature on April 16 was 42° and on April 25 it was 38°. In only three days of April was the maximum temperature above 61°. It is unlikely that materially lighter clothing is worn during April. For our present purpose April, therefore, should be classed with the winter months.

The figures just presented indicate that clothing is, relatively, a negligible factor. Were clothing an important factor, the weights for June to September would show a decrease, as compared with those for January to April, and the weights for October to January would show a considerable increase as compared with those for June to September. No such fluctuations are apparent.

Observe now the figures from March to June (table 5).

	<i>Increase in weight</i>	Pounds
March to April.....		+0.22
April to May.....		-0.16
May to June.....		+0.05

If a change from winter to summer clothing be invoked to explain the low average of growth from January to April, just discussed, it cannot be used again to explain the failure to grow from April to May, and a third time to explain the failure to grow from May to June. It will doubtless be found, taking the average of eight or nine years, that the change from winter to summer clothing is made within a short period and bears a definite relation to the temperature curve.

Finally, too much emphasis must not be laid upon hypothetical differences in summer and winter clothing. Careful enquiry shows that the indoor clothing of Boston public school children does not change during the school year as much as might be supposed. Such changes cannot explain away the periodic growth demonstrated in this investigation. With every reasonable allowance for error, it seems impossible to deny a seasonal variation in the weights of school children.

V

In figure 2 the seasonal curve departs from the present standard curve by fully 2 pounds, a difference almost three times the average total growth for the first five months of that year. Such deviations justify two deductions:

1. To determine the normal growth in weight, the child must be weighed once a month, or oftener. If the child is weighed less often, the seasonal variation will be missed.
2. True curves of growth in weight demand that the monthly weights be distributed according to the months of the year, and not according to the months or years of age, as is the present custom.<sup>3</sup>

<sup>3</sup> A substantial part of the cost of this study has been paid by the Permanent Charity Fund, whose assistance is gratefully acknowledged. I am much indebted also to the Department of Education in Harvard University for the loan of Mr. L. A. Maverick during the summer of 1919.

## CERTAIN CHANGES NOTED IN ERGOGRAPHIC RESPONSE AS A RESULT OF TOBACCO-SMOKING

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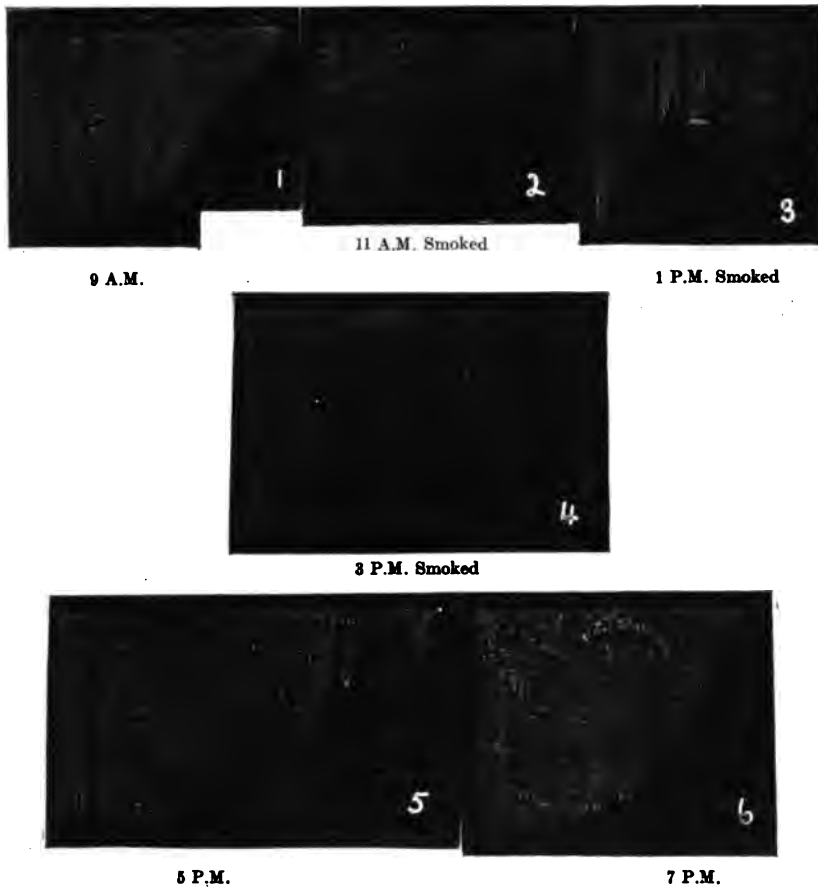
This report is based upon material secured in the course of an investigation undertaken with a view to ascertaining the effect of tobacco-smoking upon voluntary muscular work, as determined through ergographic observation. The study was suggested by Lombard's (1), (2) findings and the procedure followed was essentially that utilized by him (q.v.).

In the summer of 1915 when, for a month, I had the opportunity of association with Lombard in connection with an ergographic study (3) upon which he was engaged at the time, I noted that my own tracings were uniformly free from those periodic recoveries, after the first loss of power, which were consistently characteristic of Lombard's records (1). These recoveries, or returns of power, failed to manifest themselves in my curves in spite of any subjective awareness, or even desire, and continued physical endeavor. The records showed throughout only a gradual falling off of performance registry (fig. 1) from maximal contraction to zero. After smoking, Lombard noted that the initial loss of power came more quickly, to be followed, however, as in his non-smoking records, by the characteristic periods.

In the spring of 1917 I found it possible, personally, to undertake a short study of the effect of smoking upon ergographic response, expecting to encounter, if anything, only a diminution in total amount of work performed. Greatly to my surprise, I noted the consistent appearance of periods practically identical with those characteristic of Lombard's smoking and non-smoking records. Since that time it has been possible to elaborate the results somewhat and, in spite of their apparent slenderness, they were deemed worthy of report.

Prior to the time of undertaking this study, I had been only an occasional smoker, just able to tolerate one or two mild cigarettes with-

out suffering any of the acute tobacco effects. The specific procedure followed in this work was, as indicated, that employed by Lombard (1). The ergographic records were taken every second hour, daily, from about approximately 9 a.m. to 7 p.m., care being taken to look away from the record as it was being taken and, as far as possible, to avoid



FIGS. 1-6

all suggestive influence. After a week of preliminary work to secure adaptation to the schedule and to overcome initial practice effect, the actual smoking records were begun; i.e., a cigarette of medium strength was smoked just prior to the moment of beginning a record, at the expiration of the two-hour interval. Effort was suspended after the first loss of power.

At first, aside from an apparently marked decrease in the extent of the record, no essential change was noted. On the third day of the test, however, it was found that, if effort were continued after the first loss of power, it was possible, in contrast to the results of previous trials, to continue long beyond this point, the curve then showing distinct periodicity (figs. 2, 3 and 4), seemingly identical with that so manifest in Lombard's curves. This finding, it might be well to note, was extremely unexpected and wholly unlooked for. It was found, in addition, that this effect would persist for some hours without additional smoking (fig. 5).

When electrical stimulation was applied directly to the muscles by means of the induction current, essentially as described by Lombard in his *Laboratory Manual* (4), the weight having been materially diminished, the record showed both with and without the use of tobacco a distinctly unbroken character, marked by no period formation whatsoever, thus indicating the causal locus of the periodicity to be further central than the muscles or the nerve endings. The same findings were noted on nerve trunk stimulation also, thus in both respects corroborating Lombard's personal observations.

Four other subjects, only one of whom had previously smoked to any degree, were obtained. Although these subjects were untrained and the schedule much less thorough, the findings seemed to bear out in a general way those determinable in my own records.

It might be well to indicate, at this point, that these results seem to be at variance with observations made by Hough (5) in tests carried out upon himself, utilizing a somewhat different apparatus.

It appears, therefore, that the essential cause of the periodicity is central nervous system fatigue, or depression, induced in this instance by tobacco products absorbed in the act of smoking. On this basis it might not be impossible to account for Lombard's periodicity, for which, thus far, apparently no satisfactory explanation has been afforded. He reports having already smoked for a number of years when the periods were first noted and has since observed that although, as indicated, the first loss of power comes more quickly on smoking days, the periods always appear sooner or later, and have apparently the same character at all times. In view of the fact that the periods are present constantly, even when smoking had been dropped for a period as long as a month, it is not inconceivable that the long-continued use of tobacco may have, in this case, exercised some more or less permanent change in the substance of the central nervous system, specifically concerned in the mech-

anism of ergographic response. On the other hand, it is equally possible that the periods might have occurred even without smoking, had the central nervous system been originally of a type especially readily susceptible to fatigue. Lombard is unable to state whether any of his eight subjects, two of whom manifested periods, were smokers. In the same way it is conceivable that other central nervous system depressants may produce similar changes in ergographic response.

It is distinctly unfortunate that time and opportunity did not permit more extensive investigation, and with a larger series of subjects, both smokers and non-smokers, and it is primarily in the hope of stimulating further work that this brief note is submitted.

#### SUMMARY

On the basis of the results obtained in this study, it appears that under the influence of tobacco-smoking, a distinct periodicity may be demonstrated in the curve of ergographic response, arising apparently as a result of central nervous system fatigue or depression.

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## DETERMINATION OF THE CAPILLARY BLOOD PRESSURE IN MAN WITH THE MICRO-CAPILLARY TONOMETER

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Physiologists have long appreciated the prime significance of the capillary circulation and in recent times clinicians are coming more and more to realize that this part of the circulatory bed is of importance in connection with purely medical problems. The cardio-vascular system functions for the distribution of the blood but the effective changes pertaining to the nutriment of the tissues occur in the capillary bed. Here the metabolic interchange takes place; food is delivered to and waste is largely withdrawn from the active cells. It is, therefore, important to know the pressure under which the blood is delivered to the capillaries in different conditions of health and disease and to correlate this knowledge with the functional activity in other parts of the vascular bed. At the present time no method is adapted to such a study either in experimental animals or in the human being.

The fundamental observations of Roy and Brown (1) in 1878 on the capillary circulation in the frog were never developed for application to the circulation in the mammal because the method was inadequate to this purpose. Quite recently Cannon (2) has emphasized the significance of the capillary bed in traumatic shock, and Dale and Laidlaw (3) and Dale and Richards (4) in histamine shock. These authors conceive that in shock the capillary walls are injured by circulating poisons so that they lose tone with the result that a large volume of blood is pooled in the capillary area. This pooling of the blood in the capillaries accounts for the primary fall of arterial pressure, which is further accentuated by a transudation of plasma through the injured capillary wall so that there results an actual decrease in circulating blood volume. These two factors are regarded as the basal cause of the circulatory phenomena of shock.

Lombard (5) showed a number of years ago that if a drop of oil be placed upon the skin it is possible to see the underlying capillaries, and



he sought with this information to develop a method of determining the pressure of the blood in these vessels. For this purpose he used a small glass chamber, the floor of which was made of gold beaters' skin with a small hole in the center. The chamber was filled with glycerine and brought into contact with the skin so that it was possible with a microscope to observe through the glass roof and a small opening in the membranous floor the effects which were developed in the underlying capillaries when the pressure on that area of skin was elevated by forcing more glycerine into the chamber. Although Lombard made a number of observations with this instrument, the technique was so difficult that it was not possible of wide application. With Lombard's experience in mind, we have developed a method which we believe is applicable to the study of capillary blood pressure both in animals and in man, and the purpose of this paper is to describe our method and to give the results which we have thus far obtained with its use.

There are some twenty-five articles in the literature bearing upon the determination of the capillary blood pressure obtained by the use of various methods, and the results reported in these articles vary from 7 mm. to 70.5 mm. Hg. for the normal capillary blood pressure in man. It is obvious that if the results by the several methods vary to this extent, they cannot be considered as trustworthy. It is not surprising, therefore, that Friedenthal (6) in a recent review of the subject, reaches the conclusion that capillary blood pressure determinations can be of little practical significance. They are, in fact, of less value than inferential deductions drawn from the values of arterial and venous pressure. Inferential deductions, on the other hand, are certainly not free from objections, as is shown by the following.

Thus Fick (7) thought that pressure in the capillaries is almost as high as in the arteries, and that the greatest fall in pressure occurs in the small veins. His conclusions are based on theoretical considerations.

Following Poisselle it is generally believed that the principal loss of energy occurs in the capillaries because the blood channel is narrowest in the capillary area. On the other hand Campbell (8) has assumed that the greatest fall in pressure occurs in the small arteries and Levy (9) came to the same conclusion as the result of mathematical calculations. Goldmann (10) believes that the principal loss of energy occurs in the arteries. The lumina of the smallest arteries are not much greater than those of the capillaries. The velocity is much greater in the former, however. The result is the great fall of pressure in the small arteries.

Bargolomez (11) has thrown some light on this important question by the following experiments. He cannulated the smallest possible branches of the arteries (middle and posterior auricular arteries) and also the corresponding venous tributaries, and recorded their pressures monometrically. To facilitate the work he dilated the vessels (application of heat) and introduced needle cannulas. Then he allowed the vessels to return to their original caliber before starting his experiment. Thus he found that the normal blood pressure fall in the capillaries is very slight (about 4 mm. Hg. in the rabbit's ear). He concludes that the greatest fall in blood pressure (90 per cent) occurs normally in the arteries of moderate caliber.

It is perhaps as striking to the reader as to ourselves that a trustworthy method of actually determining the pressure in the capillaries is wanting. Let us for a moment review the previous methods employed for this purpose. The majority of them rest upon the principle, introduced by von Kries (12), that the paling of the skin is dependent upon the pressure and amount of blood in the superficial capillaries. von Kries employed a small glass plate of known size and applied it to the surface of the skin and the amount of pressure necessary to cause the skin to pale was interpreted as a criterion of the capillary blood pressure. We shall show that this criterion is inadequate in that the paling of the skin is not necessarily accompanied by a cessation of flow in the capillaries and we believe that it is due rather to an emptying of one or more of the venous plexuses lying in the dermis.

Spalteholtz (13) has published drawings of a reconstruction model of the blood vessels of the skin. These show that there are three venous plexuses in the cutis and one in the subcuticular layer. We believe that as increasing degrees of pressure are applied to the skin, these plexuses are successively more or less emptied of their contained blood with the result that the skin develops varying degrees of pallor. Our observations make it clear that even extreme grades of pallor are not necessarily associated with a cessation of blood flow in the superficial capillaries. It follows, therefore, that pallor of the skin cannot properly be regarded as evidence of capillary collapse.

The principle of skin pallor underlying the method of von Kries was likewise employed by von Basch (14), by von Recklinghausen (15) and by Basler (16). von Basch sealed a small glass capsule on to the skin and determined the pressure necessary to cause paling of the underlying area.

von Recklinghausen's method was essentially the same. It is that which he employed for the determination of the venous blood pressure. Basler used an instrument which he called an ochrometer; this consisted of two closed chambers with glass roofs and floors of gold beaters' skin which was sufficiently translucent to indicate any changes in color in the underlying skin. Two fingers were used, one to observe the degree of pallor and the other for the control. The microscopic fields were brought up to a single eye piece by means of prisms. One of the chambers was then inflated, causing the membranous floor to press upon the skin until the first evidence of pallor, as determined by the control finger, was observed.

von Kries, using his method, gives as the normal capillary pressure 37.7 mm. Hg. This determination was made when the hand was 490 mm. below the crown, a position which we assumed to be approximately at heart level. He likewise reports the capillary blood pressure in the ear as being 20 mm. Hg., and in the mucous membrane of the rabbit's mouth as 33 mm. Hg.

Hough and Ballantyne (17) employed the method of von Kries to study the effect of temperature on the capillary blood pressure. Their readings for the normal ranged from 40 to 50 mm. Hg. at a room temperature of 20-21°C. When the temperature of the surrounding air was reduced to 6°C., the capillary pressure was 65 mm. Hg., and when the temperature was raised to 26°, it was from 50 to 55 mm. Hg. From our own observations we judge that the temperature effects noted by these authors are associated not with actual changes in capillary blood pressure but rather with the behavior of the superficial venous plexus. We believe a significant factor contributing to the pallor of the skin is a decreased amount of blood in this plexus. If the latter is relatively empty and the skin is correspondingly pale, it will require a greater skin pressure to develop a further paling. The paling under these circumstances would then be due to the pressure emptying the deeper-lying plexuses. The resistance to compression of the skin vessels increases as the vessels compressed lie deeper and deeper in the several layers of the skin. It follows, therefore, that when the skin is cold and consequently pale, a greater pressure will be required to produce a further paling than would be the case if the skin were of a normal color. Hough and Ballantyne also report a heightened capillary pressure associated with arterial constriction, hence they thought that capillary pressure was dependent on some other factor than that of the arterial tone. These findings like their findings in connection with the effects of tem-

perature do not agree with our results, and we believe that the latter, as in the case of the former, are associated with the inadequacy of the criterion employed.

Natanson (18) has published two papers using the von Kries method. He took for his standard the complete blanching of the skin and studied, among other things, the effect of mass compression of the arm on the capillary pressure in the hand. Using the criterion of complete blanching of the skin, he found the normal capillary pressure to be 70.5 mm. Hg. The highest capillary pressures were observed when the constricting band exerted a pressure of 42.5 mm. Hg. When the constricting pressure was raised to 52.3 mm. Hg., capillary pressure fell. He assumes that at this pressure both the arteries and veins were being compressed and draws the conclusion that the capillaries cannot be filled with blood except under the influence of arterial pressure. If the latter fails, the capillaries become bloodless and collapse through the effect of a relative rise of tissue tension. We have not been able to confirm the latter observation, and believe that here again the inadequacy of the criterion employed by Natanson is sufficient to explain his results.

Schiller (19) and also Rotermund (20) made use of von Fries's method slightly modified by the addition of Fick's ophthalmotonometer (21). This addition served merely to make the pressure readings easy since they could be read off on a scale calibrated against a spring. Schiller found that the highest capillary pressure (about 40 mm. Hg.) occurred when the temperature of water applied to the skin was approximately that of the skin, namely, 35°C.

Rotermund, using the same method, found the capillary pressure on the skin of the forehead when the subject was in a recumbent position, averaged about 26.8 mm. Hg. He also applied this method to study the influence of age, nutritional state, dyspnea, nephritis and arteriosclerosis on capillary blood pressure.

von Basch applied his glass capsule method to a study of the capillary blood pressure in human beings and upon experimental animals. The criterion was essentially the same as that employed in the von Kries method. He found that the capillary blood pressure in the rabbit's ear did not differ materially from that of human subjects. In the rabbit's ear the pressure ranged from 21 to 25 mm. Hg., and in the healthy human being it was between 25 and 30 mm. Hg. In human beings he observed that a low capillary blood pressure was frequently associated with high arterial tension, but at times the converse of this

was true, namely, that a high capillary pressure was associated with a low arterial tension. His inference from these observations led him to the belief that the capillary pressure is independent of the arterial pressure.

When the chest of an experimental animal was compressed, there occurred a rise in capillary pressure accompanied by a moderate fall in arterial pressure. He interpreted this result as indicating that the rise of capillary pressure followed venous stasis. Similarly in man chest compression produced a moderate rise of capillary pressure with a corresponding fall of arterial pressure. The fact that the capillary pressure rose less in man than in the experimental animal suggested that the venous stasis was less extreme. He also cut the cervical sympathetic nerve in the rabbit and found an increase in capillary pressure associated with the slight fall of arterial pressure. This pointed to a dilatation of the arterioles of the rabbit's ear and was confirmed by direct inspection. The injection of strychnine caused a rise of arterial pressure with a fall of capillary pressure; in other words, the converse of the above experiment. This was interpreted to mean that a constriction of the arterioles had occurred.

von Basch formulated the following hypothesis: that the degree of capillary pressure rise may serve to differentiate between venous stasis and arteriolar dilatation. While the former raises capillary pressure markedly without altering arterial blood pressure, a condition of arteriolar dilatation produces a moderate increase of capillary pressure with a corresponding fall in arterial blood pressure. He applied these results clinically and obtained data which he regarded as of distinct value in both diagnosis and treatment.

Using Hooker's (22) capsule with the criterion of paling, Briscoe (23), in a study of the Raynaud phenomena, in cases of "irritable heart," found that when the hand was cyanotic the capillary pressure was 36.9 cm. of water as compared with the normal controls which gave a pressure of 23.5 cm. of water. In individuals subject to vasomotor changes when the color of the hand was normal, the capillary pressure was 25.3 cm. of water, and that when the same hand was blue the capillary pressure was increased to 33.3 cm. of water.

von Recklinghausen gives as the normal capillary pressure in the finger tip with the hand at heart level, a reading of 52.5 mm. Hg.

Basler's ochrometer has been used by Basler himself and by a number of other observers. The normal capillary pressure as given by Basler is about 7 mm. Hg. Goldmann, using this method, concludes

that the normal capillary pressure is about 8.5 mm. Hg. A moderate rise in external temperature causes no appreciable change in the capillary pressure, but if the external temperature be raised 10°C. or more, the capillary pressure is increased.

Landerer (24), using Basler's ochrometer, found that the normal capillary blood pressure ranged from 17 to 25 mm. Hg. During a cold bath the capillary pressure fell while the arterial pressure rose. In a warm bath the capillary pressure was unchanged while the arterial pressure fell. Landerer, Krauss (25), Friedenthal and others have used this method in clinical cases.

Krauss studied the capillary pressure in circulatory disturbances (valvular disease, myocardial disease, venous stasis), pulmonary diseases (emphysema, chronic bronchitis, tuberculosis), status asthenicus, severe anemia, carcinoma and cachexia. Besides the ochrometer he also used an apparatus of his own (a microscopic method) and also Weiss' blood method. He obtained some very interesting results. In brief, they confirmed the older observations of von Basch and Roter-mund and agreed with those of Landerer.

The foregoing methods are all based upon the principle of paling of the skin originally introduced by von Kries. A decidedly different method has been employed by Basler and by Weiss. Weiss's (26) method consisted of pricking the skin and observing the pressure necessary to stop the exudation of blood through the wound. The finger tip was enclosed in a chamber containing fluid in which the pressure could be raised. It was necessary, of course, to insure that bleeding continue after the pressure was removed.

Basler (27) pricked the volar surface of the middle finger with a needle; he then sealed a piece of rubber tubing onto the finger (by applying a hot rod against the edge of the rubber). This formed a little chamber in the center of which was a bleeding spot. The chamber was filled with hirudinized saline solution, and closed with a roof. Now the pressure of the exuded blood was registered manometrically. His recorder was of the lever type and rather delicate. He named this apparatus the "Hautmanometer." The determinations made with this apparatus agreed quite closely with those of the ochrometer.

Both of these blood methods are faulty because it is not possible to know the depth of the wound, since the skin thickness is so variable and consequently the size or the depth of the vessels punctured is a matter of chance.

The theoretical objections to the various methods above discussed together with the fact emphasized by Friedenthal that the readings obtained by these methods vary by more than 100 per cent indicate clearly that they have little or no practical application. In order to be sure that the readings accurately represent the pressure in the capillaries themselves, a criterion similar to that employed by Roy and Brown, namely, the visual determination of the point at which the capillary flow ceases, is essential. Using a tonometer similar to that which we have employed and the criterion of cessation of corpuscular flow, these authors obtained pressure readings in the capillaries and venules of the web of the frog's foot of 7.3 to 11 mm. Hg.

Lapinski (28) employed the Roy-Brown method to study the effect of nerve section on the capillary pressure in the frog. He obtained no very clear-cut results; however, his determinations of normal capillary blood pressure in small frogs was between 15 and 44 mm. Hg. and in large frogs between 30 and 60 mm. Hg.

Natanson, in his studies on the effect of mass ligature, controlled his observations on man by experiments on the frog. He found the normal capillary pressure varied from 12 to 24 mm. Hg. in the different capillaries of the frog's web.

That such a criterion may be applied in the case of the human subject is indicated by the observation of Weiss (29) who, using oil on the skin as suggested by Lombard, first observed corpuscular flow in the human capillaries. We have developed an instrument applicable to man and experimental animals which permits of the accurate use of this criterion.

If a drop of oil be placed on the skin of the hand and the area so treated be examined with the microscope under a strong light, as, for example, a 40 Watt electric bulb placed close to the microscope objective, one may readily see the capillary tufts scattered through the field. Most of these tufts come up from the deeper parts of the dermis, fold over and return so that only a small part of the capillary loop can be brought into focus. Under such conditions it is scarcely ever possible to see the corpuscular flow. Occasionally a capillary may be found which, arising from the depths of the dermis, turns at right angles and takes an horizontal course for a short distance, and in such a case, under favorable conditions, the corpuscular flow may be observed. Such capillaries are so rare, however, on the general surface of the skin, that they do not serve for pressure determinations. In the skin overlying the matrix of the finger nail however, many capillaries take such an

horizontal course and in a single microscopic field a number of these vessels may be brought into focus at one time. Here one frequently sees corpuscular flow without offering any resistance to the blood stream. If a constricting pressure be applied to the upper arm the corpuscular stream may be seen in many of the capillaries without difficulty. A drawing of the picture seen is given in figure 1. The sharply outlined capillaries are seen as red threads lying on a pink ground. The picture is very much clarified if the skin be first scrubbed with soap and water and afterwards thoroughly dried. This procedure removes the loose epidermis and softens the tissues.

The methods based on this principle introduced by Lombard are the Krauss method, Basler's kapillar-tonometer (30) and Kylin's method (31).

In Krauss's method the capillaries are visualized through a simple lens (magnifying 10 $\times$ ). Krauss takes as his criterion the disappearance of the capillaries. We have employed such a method but have not succeeded in making the capillaries disappear at pressures corresponding to those of Krauss. We believe that the paling of the skin (which occurs at very low pressures) and the minute size of the capillaries as seen at such a low magnification may give the impression of a disappearance of the capillaries when even slight pressure is applied. When controlling this method with our own (in which a magnification of at least 68 is used) we have been able to see distinctly capillaries which were hazy and almost invisible with a magnification of ten.

Concerning Basler's tonometer we may say that except for the fact that the gold beaters' skin has no circular opening in its center, it is almost identical with that of Lombard. The glycerine reservoir and the fact that the chamber is filled with glycerine, is common to both methods. An added difficulty in the method of Basler may be the collection of air bubbles within the chamber which will disturb the clarity of vision. In our experiments in the early part of 1919 we used chambers which were almost the counterpart of Basler's, but have found them difficult to work with and for reasons of practicability have discarded them. Basler's criterion is the disappearance of the capillaries. We believe this to be incorrect in principle and difficult of execution in the majority of instances. In the publication dealing with this method he gives no results obtained by its use.

Kylin's method, which was presented at the Ninth Nordiske Congress of Internal Medicine held at Copenhagen, August, 1919, depends on the visualization of the capillaries. Unfortunately we have been unable to



obtain any exact information about his method, his criterion or his results.



Fig. 1. A drawing of the finger tip to show the area of skin at the base of the finger nail which is used in the determination of the capillary blood pressure. The circle overlying this area represents the microscopic field when the skin is observed under a drop of oil with the aid of a strong light. The capillaries are slightly diagrammatic: a single focal plane will not bring them all into focus at one time.

*The micro-capillary tonometer.* The instrument which we have used is shown in figure 2. It consists essentially of two adjusting devices. Screw 1 permits of raising and lowering the finger rest so that the area of skin under observation may be brought into an horizontal plane. Screw 2 permits of adjusting the pressure capsule in suitable contact with the skin. The finger rests on plate 3, which rocks so that the finger tip may assume a comfortable position. The instrument is placed on a microscope stage and when the forearm is supported with a comfortable rest, the subject is sufficiently comfortable, so that there

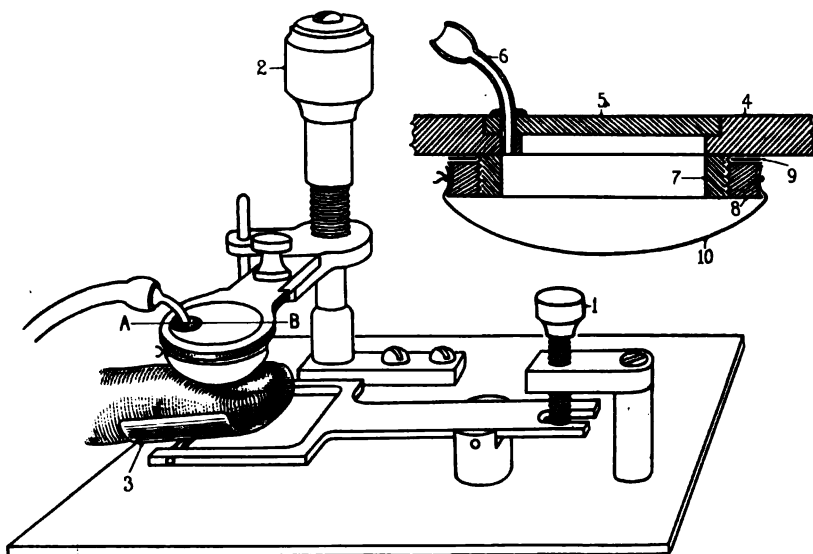


Fig. 2. The micro-capillary tonometer. The instrument is of such a size that it rests on the microscope stage. Description in text.

is no movement or tremor of the finger. The pressure capsule is shown in the insert figure, which represents a cross section along the line *AB*. It consists of a brass ring, 4, into the upper surface of which is sealed a thin glass plate, 5. Through the latter passes tube 6, which serves for the inflow and egress of air. To the lower surface of plate 4 is attached a brass ring, 7, threaded on the outside to receive the collar, 8. The latter screws air-tight against the washer, 9. Over the brass collar 8 is tied the gold beaters' skin, 10. The metal tube, 6, is connected through a rubber tube with a rubber ball of about 200 cc. capacity, which may be compressed between two plates by means of

an adjusted screw, a device similar to that which is ordinarily used for calibrating the Hürthle manometer. A bypass on the tube connecting the capsule with the rubber bulb leads to a single arm mercury manometer, by means of which the pressure in the chamber may be determined.

After the finger has been brought to a comfortable position on the finger-rest with the area for study in the horizontal plane, the pressure capsule is lowered until the gold beaters' skin comes softly in contact with the upper surface of the finger. The gold beaters' skin is tied on sufficiently loosely so that when the air is forced into the capsule it exerts pressure upon the skin without loss of pressure due to the tension on the membrane.

*Preparation of membrane.* The successful use of this device depends upon obtaining gold beaters' skin which is free of porous openings, is entirely soft and pliable and is transparent. We have found that gold beaters' skin prepared according to the following directions fulfills these requirements. The membrane is first washed in tepid water to remove all powder and dust which may be adherent to the surface. This cleansing is facilitated by rubbing both surfaces of the membrane with the ball of the finger or with a piece of absorbent cotton. It is then rinsed several times in clean water, after which it is ready for use. The brass collar, 8, in figure 2 is now removed from the instrument and screwed down over an obturator which, presenting through the collar, has an oval contour such that when the membrane is tied into position it will lie somewhat as shown in the detail. With the collar in position on the obturator the membrane is laid over the top and tied securely with several turns of silk thread. The collar is now returned to the instrument, when it may be tested to see whether or not the attached membrane is perfect. If it stands a pressure of 50 mm. Hg. without leak, it is suitable for further treatment.

The collar with the attached membrane is now drained free of water and placed in pure glycerine for 24 hours, after which it is removed, drained free of glycerine and placed in castor oil. The preparation should be left in castor oil for at least 24 hours and may be left in the oil indefinitely. It is thus possible to prepare a number of membranes at one time and to keep them in castor oil until such time as they may be required.

When a membrane is required for use, the ring to which it is attached is removed from the oil and screwed into position on the instrument, a test being again made to be sure that the chamber is air-tight. If a

leakage occurs at this point it is probably due to an imperfection in the washer.

If now the adjustment to the finger be made and the area of skin beneath the member be observed with the microscope, it will be seen that the membrane is entirely transparent so that there is no difficulty in observing the underlying capillaries. Failure to see the capillary field plainly indicates that the membrane is clouded, in which case it should be discarded. This condition is not likely to be found when using a new membrane. A membrane, however, which has been used repeatedly and exposed to air and dirt is liable in the course of time (weeks) to deteriorate and if there is any difficulty in obtaining clear vision of the field it is probable that the transparency of the membrane is at fault. When the instrument is not in constant use it is advisable to return the membrane to castor oil. With reasonable care a membrane should last indefinitely.

A second technical requirement is that the skin should be clean and free from moisture. It should be scrubbed lightly with soap and water and thoroughly dried. The oil on the skin which makes the underlying capillaries visible probably serves by its intimate penetration of the epidermal layer to do away with light reflection from the uneven surface. If then moisture intervenes at the oil-skin boundary, the field is less distinct. It sometimes happens in prolonged observations that the clarity of the field is lost. This may be due to the excretion of sweat. If the oil be wiped off and the finger be thoroughly dried a fresh drop of oil will make the field as clear as before.

In using the instrument we have found it desirable to employ a microscope giving a magnification of approximately  $70\times$ . The essential point is to magnify enough to readily visualize the movement of the red cells when the stream is slowed without undue loss of definition and depth of focus. Leitz objective 3 and ocular 1 fulfill these requirements. To use such a lens combination with a rather short working distance, the skin with the overlying oiled membrane must be brought quite close to the glass roof of the chamber. Care must be exercised, therefore, that in manipulation the membrane shall not touch the glass. If this occurs one must dismount the collar and clean the glass, otherwise the capillary picture will be clouded. It is of distinct practical help to one using the instrument for the first time, to first become familiar with the location and appearance of the capillary bed under the microscope. When this is done it is a simple matter to locate the proper field with the chamber in position.

We have experimented with chambers of different diameters but have found that this is a matter of no consequence in obtaining correct readings and we now use for routine observations a chamber of such a size that the brass collar is 25 mm. in diameter. In order to insure that the pressure within the chamber is all transmitted to the underlying skin, it is advisable to make several determinations of the capillary pressure with the chamber at slightly different vertical positions. The position of the chamber which gives the lowest capillary pressure reading is the correct one. With a little practice one makes this adjustment readily and there is no necessity for such preliminary observations. The purpose is, of course, to insure that none of the pressure is lost in the resistance offered by the membrane itself.

*Corpuscular flow.* With the chamber in position over the finger, when there is no pressure exerted on the underlying skin, the picture is frequently disturbed by reflections of light from the folds of the membrane. This disturbing feature is at once removed if a pressure of 2 or 3 mm. be applied, a procedure which has the effect of smoothing out the membrane over the surface under observation. When the area is first observed it is uncommon to obtain any indication of corpuscular flow. If now the pressure within the chamber be slowly raised by compression of the rubber bulb, the corpuscular flow becomes evident and the red cells are seen streaming slowly through the capillary. As the pressure is further raised this corpuscular flow becomes slower and slower until there is no further continuous forward movement of the corpuscles. At this time one frequently sees a to-and-fro movement of the corpuscles without progress. At first thought this phenomenon would seem to be associated with a transmitted pulsation from the underlying vessels. It may however be due to rhythmic contractility of the capillary endothelium. As the pressure is further raised, this to-and-fro movement of the corpuscles ceases. Now a further increase in the pressure acting on the capillaries may cause the corpuscles to travel in a reversed direction, that is to say, from the venous side toward the arterial side. We are able to offer no satisfactory explanation of this fact at the present time. If now the pressure be slowly lowered the reversed flow ceases and the corpuscles again stand still. Then the to-and-fro movement of the corpuscles is seen, and finally with a further lowering of the pressure the corpuscles stream forward in the normal direction. The pressure at this instant is taken to represent the capillary blood pressure and our reading is therefore made at this point.

The pressures at which the above events occur in one and the same capillary are depicted in figure 3 and again for a second subject in figure 4. The plotted lines in each figure represent several observations on a single capillary. Note that the skin pales in both subjects at a pressure of about 8 mm. Hg., while the corpuscular stream is not slowed until the pressure has been raised to 18 mm. Hg. in one case (fig. 3) and 27

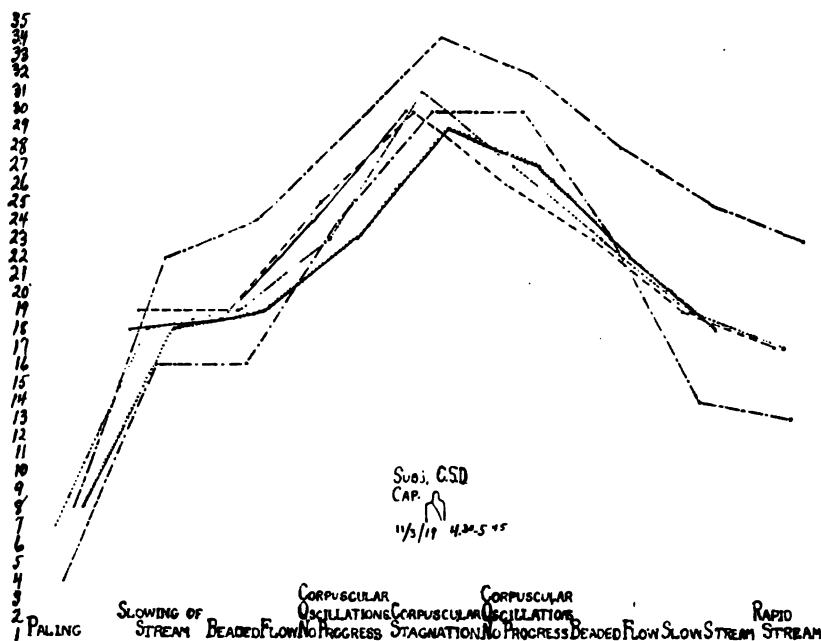


Fig. 3. To illustrate the pressures at which the several events in capillary blood flow occur. The figures along the abscissa represent pressure in millimeters of mercury. The ordinates divide the chart according to the observed behavior of the corpuscles with an increasing and then with a decreasing pressure. All the observations were made on one capillary, the several plotted lines representing single observations.

mm. Hg. in the other (fig. 4). The slowing of the corpuscular stream undoubtedly represents the condition when the pressure without approximates the pressure within the capillary. It is clear, therefore, that paling of the skin is a wholly inadequate criterion of capillary blood pressure. These figures indicate further that repeated observations of the same capillary agree closely in the pressures at which the events under discussion occur. Experience has taught us, however, that the

slowing of the corpuscular stream which occurs with a rising pressure is much less readily appreciated than is the quickening of the stream which occurs with a falling pressure. Consequently we have found it expedient to raise the pressure until the forward movement of the corpuscles ceases and then to lower it, giving close attention to the behavior of the corpuscles. As the pressure falls the corpuscles at first progress slowly and with a further slight lowering of the pressure there is a sudden sharp acceleration which serves as a sharp criterion and

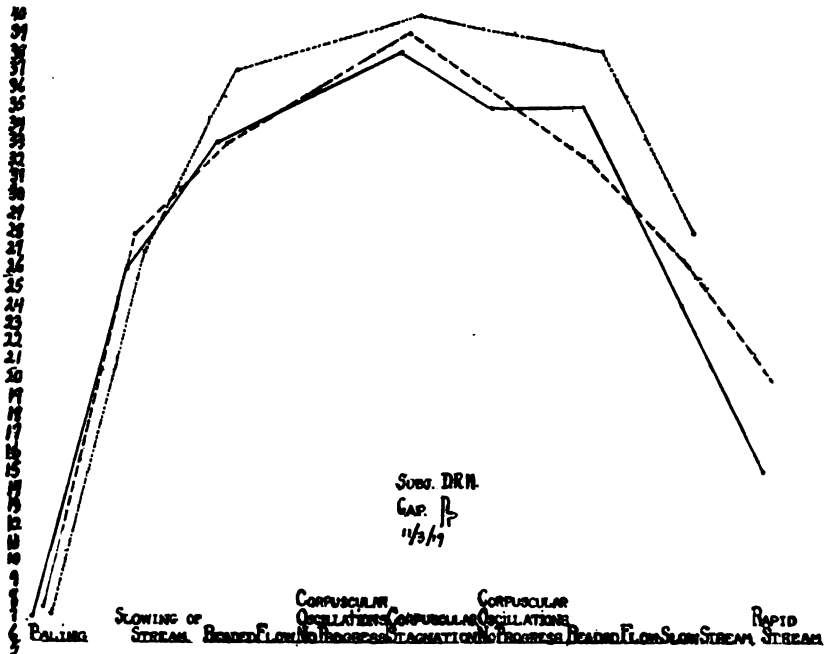


Fig. 4. See figure 3.

one which is easily recognized. The pressure then represents what we have taken to be the capillary blood pressure.

During the procedure above outlined, the capillaries are sometimes emptied of red blood corpuscles, but more often this is not the case, that is to say, when the pressure applied to the skin is sufficient to stop completely the corpuscular flow, the cells are still present in the capillary loop. This observation goes to show that the assumption made by von Kries and other earlier observers that the paling of the

skin is associated with an emptying of the lumina of the capillaries is incorrect. Furthermore, one may readily appreciate that the paling of the skin associated with the application of pressure becomes very extreme even before the corpuscular flow is distinctly slowed. Observations of this kind have led us to the conviction that the criteria used by most other observers are wholly inadequate to represent true capillary blood pressure.

It frequently happens that when one obtains a focus of the capillary field, one or more of the capillaries will stand out conspicuous and large. Such capillaries represent vessels in which the corpuscular flow is stagnated. The individual corpuscles in such a capillary cannot be made out; apparently they are thickly packed together. The pressure may be raised very considerably without changing in any way the appearance of such a vessel. This fact accords with the original observations of Roy and Brown which have been abundantly confirmed by later observers, that all the capillaries in a given vascular bed are not necessarily functioning at the same time.

The idea that not all of the capillaries are functioning at all times is indeed not a new one. Worm-Müller (32) in 1873 in studying the influence of blood volume on the arterial pressure comes to the following conclusion: "Under normal conditions (most likely in every part of the body) a large number of empty or poorly filled capillaries stand ready to respond to the needs of the blood stream." He also spoke of the dilatation of the capillaries resulting from the transfusion of blood. He found on post-mortem examination that the arteries and veins were not overfilled after transfusion. He therefore assumed that the blood collected in the capillaries.

Heubner (33) in 1907 saw many new capillaries open up as the result of the injection of gold sodium chloride into frogs, rabbits, cats and dogs. The experiments on the frogs are described in greatest detail. In these he looked at the frog's mesentery microscopically and injected an amount equivalent to 0.25 mgm. of metallic gold and in one-half to one minute saw the animal practically bleeding into its capillaries. He estimates that the number of capillaries visible after the injection is three to four times as many as before.

Concerning Dale and Laidlaw's work little can be added to what is so generally known about their researches on "Histamine Shock." By a series of ingenious experiments these workers have attempted to prove *a*, the active functioning of the capillaries; *b*, the independence of the capillaries from the rest of the vascular system; *c*, the capillo-



dilating effect of histamine. They were led to these problems by the discrepancy between the effect of histamine in the intact animal and on the excised arterial or uterine strip. In the former it lowered the blood pressure and in the latter it increased the tone of involuntary muscle strips. Dale and his co-workers explain the marked fall in arterial blood pressure which occurs in the intact animal and which cannot be due to a relaxation of arterial tone since histamine causes the isolated artery to contract, as due to a specific dilator action of histamine on the capillaries. Accordingly the capillary beds throughout the body are flooded and the circulating blood is insufficient to fill these areas and at the same time sustain the arterial pressure in spite of a concomitant increase of arterial tone. Whereas the experiments are very suggestive the crucial experiment, namely the effect of histamine on the capillaries as observed directly (through the microscope) is as yet lacking.

Krogh (34) has thrown considerable light on this subject by counting the number of capillaries in a definite area of muscle tissue in the resting state and immediately after exercise. He examined both fresh and fixed tissues. He found that the working muscle showed many more capillaries than the resting tissue. Hence, the conclusion that many new capillaries open up when necessary (e.g., in the working state).

It is obvious perhaps that we avoid the term capillary collapse. We have done this because of a good deal of experimental evidence (particularly that of Roy and Brown) which indicates that capillaries do not collapse when compressed. Why then do we not take capillary emptying (i.e., when corpuscles travel back from capillaries into the arterioles) as the criterion? The reason is that in a number of capillaries it is necessary to raise the pressure acting upon them much above that which will cause the corpuscles to stagnate. Hence the readings would be much too high. This is due to the fact that blood is piling up in the patent capillaries as each neighboring one is emptied of its contents (von Recklinghausen). We believe also that as the externally applied pressure rises the arterioles and capillaries are being simultaneously compressed. On this account pressure determination in a great many capillaries may be impossible with the methods of Lombard, Krauss or Basler. Krauss himself admits this difficulty. The impression that we have received from Doctor Lombard personally concerning some of the drawbacks of his method are to the same effect.

TABLE 1  
*Observations of the capillary blood pressure in normal individuals*

SUBJECT	AGE	HEIGHT	WEIGHT	+ OR - IDEAL WEIGHT	READINGS	CALCULATED HYDROSTATIC DIFFERENCE	CAPILLARY PRESSURE (AVERAGE)	REMARKS
			pounds		mm. Hg.	mm. Hg.	mm. Hg.	
1	20				20, 22		21.0	
2	21	5' 8"	150	- 4	20, 20, 18	-1.2	19.0	
3	21				23, 23, 19, 18, 22		21.0	
4	21	5' 8"	135	-19	22, 26, 25, 26, 24, 26, 28, 26	-1.6	25.0	
5	22	5' 11"	135	-25	27, 27, 28, 24		26.5	
6	22	5' 6"	150	+ 7	23, 25	-2.2	24.0	
7	21	5' 10½"	150	-18	17, 18, 31, 16, 18		18.0	
8	21	5' 10"	158	- 7	20, 22, 19, 23, 18, 24, 25, 25, 25	-1.4	22.3	15, 17 High Art. Pr. 150-180
9	22				17, 22.5, 23.5		21.0	
10	22				25, 23.5, 23.5, 22.5		23.5	
11	22				25		25.0	16, 15
12	24				27, 26, 24, 23, 23		24.5	
13	25	5' 8½"	155	- 2	22, 18	-2.2	20.0	
14	26	5' 3"	110	-17	19, 22, 21, 21, 24, 22		21.5	14, 15
15	26	5' 4½"	140	+ 5	22, 23, 19, 21, 23, 24, 24, 23, 22, 22, 22, 23, 25, 26, 27	0	23.0	
16	31	5' 10½"	158	-10	24, 27, 27.5, 28		26.5	
17	31	5' 10½"	150		22, 24, 19, 21, 19		21.0	
18	33	5' 1½"	135	+17	19, 23, 24	0	22.0	
19	34				24, 23, 22, 21		22.5	

20	47	5'	8"	140	-14	20, 21, 22, 22, 18, 22	21.0	Membrane was cloudy that day and these subjects were difficult to work with  Subject difficult to work with; cap. seem to disappear from view very readily. Subject has cold  Subject has a cold. These readings taken in early part of these studies. Technique?
21	42	5'	7"	135	-13	24, 20, 23, 23, 22, 24, 25, 23, 24, 26 24, 22, 22, 22, 24, 23, 26, 23, 22, 23	23.	
A	22	5'	4"	155	+23	35, 30, 30	32.0	
B	30	5'	10"	157	-8	27, 32, 27	29.0	
C	21	6'	0"	195	+19	12, 13	12.5	
D						15, 15, 14, 15.5, 15, 15, 15, 13	15.0	
I	16‡					22, 16, 16, 18, 18	Corrected 18.0	
II	11					14, 16, 15, 11.5, 11	17.5	
III	11					18, 16, 15	20.0	
IV	10					19, 20, 17	23.0	
V	8					15, 18, 20	26.0	Subject to paroxysms of acro- cyanosis
VI	8					16, 19, 16	25.0	

Because some capillaries exhibit no corpuscular flow and because there is an appreciable variation in the pressure in several capillaries in a given area, we have found it expedient to make pressure determinations in five or more capillaries and to average the results. This will minimize the possibility of striking capillaries of extreme pressures. The majority of our determinations, therefore, represent such an average.

*Capillary blood pressure in normal individuals.* Twenty-five adults and six children were studied. All of the determinations were made with the subjects in the sitting posture, with the hand a little below the heart level. The average capillary pressure in our series was 22.2 mm. Hg. The ages of our subjects ranged from 8 to 47 years. Of the subjects studied the lowest average capillary pressure was 17.5 mm. Hg., the highest was 26.5 mm. Hg. These results are given in table 1.

Four of our cases (A, B, C, and D in table 1) cannot be grouped with the remaining ones, because of the existence of infections (colds) in two of them and on account of technical difficulties in the other two which occurred at the inception of the work.

In some of the cases the distance between the level of the hand and the heart level was determined. Like von Kries, von Recklinghausen and Goldmann, we found that capillary pressure varies with the distance between the hand and the heart level. The difference in the pressure readings taken when the hand is at heart level and when the hand is above or below the heart level does not, however, correspond to the hydrostatic difference in these positions. This is clearly brought out by the following table given by von Kries (35).

HAND CHANGED FROM HEART LEVEL TO	DIFFERENCE IN CAPILLARY PRESSURE	CAP. PRESS. DIFFERENCE HYDROSTATIC DIFFERENCE
	mm. Hg.	per cent
205 mm. below heart level	65	33
285 mm. below heart level	116	40
350 mm. below heart level	225	64

The hydrostatic factor is thus a disturbing element in the determination of capillary blood pressure at the present time. It is therefore advisable that determinations of this pressure should be made at heart level although the inconvenience of such a procedure is considerable. Our readings unfortunately do not conform to this specification, nevertheless the range between the determinations on different subjects is remarkably small. In the case of children our readings run appreciably

lower than in adults but when correction is made for the hydrostatic factor the results correspond closely with those obtained in older individuals. Similarly the results in the group of adults show no relationship between age and capillary pressure. We have not noted in the table whether the subjects were men or women because here also we found the sex factor to be of no significance. On the whole, therefore, the data which we have thus far collected point to a very remarkable constancy in the capillary pressure in normal individuals of both sexes regardless of their ages. Krauss also found that the capillary pressure in children was practically the same as in adults.

Concerning the readings obtained in the individual capillaries, it may be seen that they run within fairly close limits. There are, however, capillaries whose pressure determinations are definitely outside of these limits. We do not average these extreme ones with the rest. The capillaries of higher pressure may be the ones lying deeper down in the skin or those of larger bore. Possibly they represent the piled-up blood in the patent capillaries resulting from the emptying of the neighboring capillaries, as suggested by von Recklinghausen. We discard very low readings when they occur in but one or two capillaries. Theoretically these are the most accurate. For practical reasons, however, we have arbitrarily eliminated these infrequent (low pressure) capillaries and have adopted instead another criterion which is quite as accurate as the former, but more easily carried out. That series of six or more capillaries of the lowest pressure is taken, in which the difference between the extreme capillaries of the series is not more than 6 or 7 (less is preferable). We warn against averaging capillaries of very low or very high pressures with the series. Let us take a concrete example.

Capillary pressure in separate capillaries....	{	12	eliminate—too low
		13	
		19	
		21	
		25	
		20	
		23	
		23	
		32	eliminate—too high
		20	
	{	38	eliminate—too high
		41	

Thus the series consists of.....	{	19
		21
		25
		20
		23
		23
		20
		<hr/>
		$\frac{151}{7} = 21\frac{4}{7}$ mm. Hg. = average

Our figures for normal capillary blood pressure (with the subject in sitting posture, hand below heart level) accord with those of Lombard (18 to 22 mm. Hg.), Landerer and Krauss. The latter two investigators (using Basler's ochrometer) obtained readings varying from 17 to 25 mm. Hg. on the normal individuals.

In our table we have also put down the difference between the actual and the ideal weight of the subject with the idea of noting the effect of over- or under-nutrition on the capillary pressure. Thus far we have nothing definite to say on this subject.

*The effect of temperature on capillary pressure. Cold.* Cold towels with pieces of ice placed between the layers were wrapped around the arm. The cold was not directly applied over the capillary area, hence the results are not to be interpreted as the direct effect of cold on the capillaries themselves but rather as a reflex effect. The duration of the application was 15 minutes. The results are graphically presented in figure 5. Two neighboring capillaries represented by the continuous and the broken lines were studied. Immediately on the application of cold there was a definite drop in the capillary blood pressure. When the cold was removed a very prompt rise of capillary blood pressure, which was maintained for about 13 minutes, occurred. Then there was a marked drop to a point slightly above normal, then another rise and finally a fall. The occurrences after the removal of cold represent the reaction after such a procedure.

It is striking how closely the curves of the individual capillaries follow each other. Only at one point do they diverge. The explanation for this would seem to be a change in the lumen of one of the capillaries independent of that of the arteriole since both capillaries were in all probability supplied by the same arteriole.

*Heat.* Heat was studied by means of an electric pad which was wrapped around the arm and kept on for 26 minutes. We made observations on six neighboring capillaries. The electric pad was set so as to create moderate heat. The effect produced by the application

of heat is also shown in figure 5. Almost immediately there was a rise in capillary blood pressure which was maintained throughout the period during which heat was applied. Upon the removal of the pad the pressure in the capillaries fell to a point below the normal value. Soon the pressure average in the six capillaries observed was as before the experiment. One is amazed at the striking parallelism of the pressure curves of the whole group of capillaries. Only here and there does a disparity become evident. For example, in the last phase two capillaries show a fall in pressure while two others show a simultaneous rise in pressure,

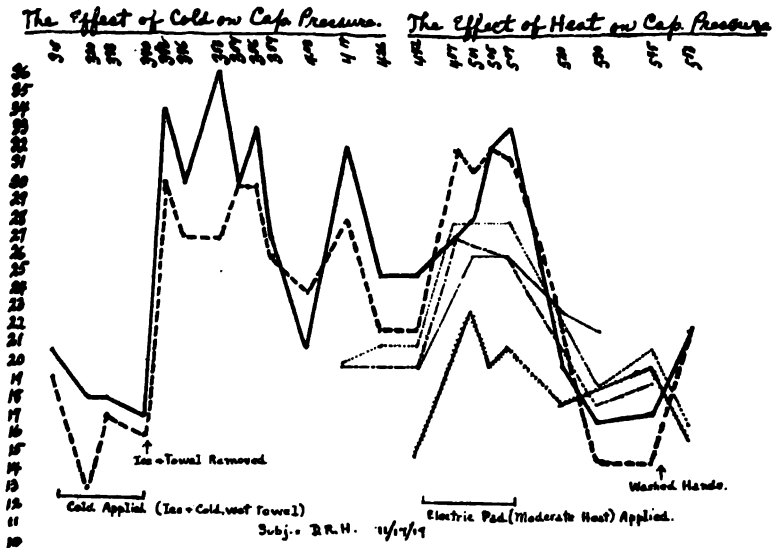


Fig. 5. To show the effect of cooling and warming the fore-arm on the capillary blood pressure in the finger. Two capillaries were followed in the application of cold and six in the application of heat.

although the average in the whole series is practically normal. Here again we must assume a contraction or dilatation of the capillaries themselves. There were occasions when we felt that the diameter of the capillaries underwent changes in the course of our observations, yet most of our evidence supporting the idea of capillary contractility is indirect and sometimes necessarily assumed. In the heat experiment the electric pad was not placed directly over the capillaries but over the forearm and hand. Here again the results are due, therefore, to a reflex effect.

In these experiments the capillary pressure responded to the application of heat and cold as if the stimulus produced vasodilatation and vasoconstriction of the feeding arterioles. We assume this to be a reflex effect from the spinal centers. Krogh (36), however, is reported to have shown that contraction of the capillaries may be evoked by reflex stimulation after the sensory nerves have been cocaineized, an observation which indicates an axon reflex. In our experiments, however, the stimulus was applied at some distance from the field of observation so that it seems improbable that the results are due to axon reflexes. The suddenness with which the capillary pressure changes, particularly after removal of the stimulus, excludes the hypothesis that the results might be due to differences in the temperature of the blood. It would be possible to differentiate between a true reflex and an axon reflex effect if the observations were repeated on a subject with impaired cutaneous sensibility.

The figure (fig. 5) shows a remarkable over-compensation of the capillary pressure after the stimulus is removed. Within three minutes after the cold was removed the pressure rose from 17 to 32 mm. Hg. and remained at this high level for a period of 15 minutes, after which it began to fall. Thirty minutes later it was still above the original value. A similar though less striking effect is seen to follow the application of heat. These changes in capillary pressure undoubtedly occur when the temperature of the surface of the whole body is altered. We have no notes indicating a definite change in the caliber of the capillaries under such conditions although, of course, when the capillary pressure was low the corpuscular stream was frequently seen without the application of external pressure.

Our observations correspond pretty well with those of Goldmann. The relative crudeness of his method, however, prevented him from investigating it in such detail as has been done here. His readings (taken with Basler's ochrometer) were definitely lower than ours.

Hough and Ballantyne, who studied the effect of temperature on capillary pressure by means of the von Kries method, found that when the temperature of the external air was reduced from 20°C. to 6°C. the capillary pressure rose from 40 or 50 mm. Hg. to 65 mm. Hg. This was probably due to the constriction of the more superficial vessels and to the fact that the first noticeable color change in the skin was produced when the deeper vessels were compressed. This again illustrates the danger of working with a method depending on skin pallor. This error is clearly the result of the fallacy of the method.



When the temperature of the external air was raised to 26°C. the capillary pressure was 50 or 55. In other words, very little (if any) change occurred. Here again the inadequacy of the method becomes evident.

Schiller also studied the effect of temperature on capillary pressure by means of the modified von Kries method (with Fick's ophthalmotonometer) but his results are difficult to interpret because of the reasons given above. Briefly they are as follows: the highest capillary pressure (40 mm. Hg.) occurred when the temperature of the externally applied water was nearest to that of the skin (30 to 35°C.). Landerer (working with Basler's ochrometer) found that the capillary pressure fell during a cold bath, while it was unchanged in a warm bath. Krauss (using his own apparatus, modified after Lombard) found that

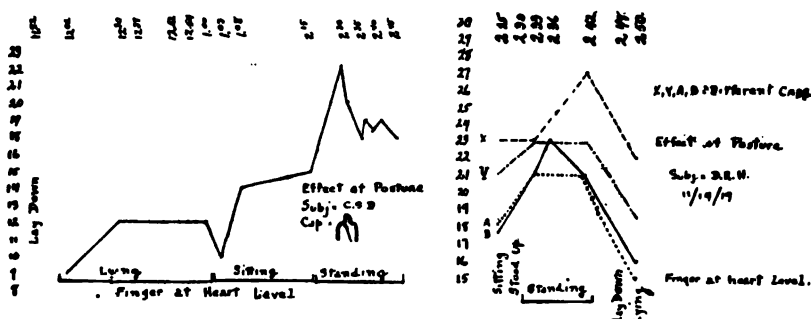


Fig. 6. Effect of posture on capillary blood pressure. Finger was at heart level throughout so that hydrostatic factor is excluded. The numbers at the left indicate pressure in millimeters of mercury.

the application of ice to his arm produced a fall of capillary pressure amounting to 20 mm. water and a rise of arterial pressure of 15 to 20 mm. Hg.

*The effect of posture.* The effect of posture on the capillary blood pressure is shown in figure 6. The hand was held at heart level in these observations in order to eliminate the hydrostatic effect. Hence our results represent the true postural effect. It will be seen that the lowest pressure occurs in the horizontal position, the highest in the vertical, and the capillary pressure in the sitting posture is midway between the two. Here again we see in the figure the individuality of some capillaries. There are two pairs of capillaries which differ from each other, yet the individual members of each couple act in close harmony as is evidenced by the parallelism of their pressure curves. Again the contractility of the capillaries suggests itself.

From this experiment it may be seen that capillary pressure determinations will vary according to the posture of the subject during the examination, although the hand be maintained at heart level. This has been impressed rather strongly on us very recently in connection with our clinical work on the bed cases in the hospital. In a number of these patients (in whom the capillary pressure was probably normal) readings ranging from 13 to 15 mm. Hg. were commonly obtained. These, of course, are a good deal lower than our original figures, which were made with the subject in the sitting position with the hand slightly below the heart level.

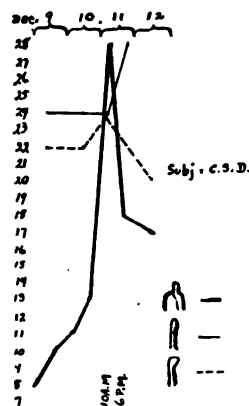


FIG. 7

Fig. 7. To show the variations in pressure in individual capillaries from day to day. While the pressure in an individual capillary may thus alter from day to day and from hour to hour, the average pressure in a group of capillaries is remarkably constant. See tables 2, 3 and 4.

Fig. 8. To show the effect of changes in intrathoracic pressure on the capillary pressure. Two observations on raising the intrathoracic pressure (left) and two on lowering the intrathoracic pressure (right) are given.

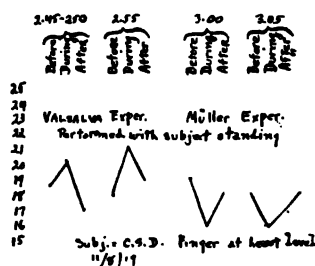


FIG. 8

The following represents pressures in a series of capillaries studied on two consecutive days.

*Diurnal variations.* Hans Friedenthal says that capillary blood pressure varies by many hundreds per cent during the same 24 hours. In our experience this has never occurred. We have found on the contrary that the average pressure of a series of capillaries varies within relatively narrow limits from hour to hour, or even day to day. Some capillaries which we have followed in this manner have shown practically constant pressures, while others have shown very great variations.

This tendency for the pressure to vary in an individual capillary from day to day is illustrated in figure 7. The figure represents the pressures found in three different capillaries on four successive days. However much the pressure may fluctuate in an individual capillary there is nevertheless a remarkable uniformity in the results obtained when the pressures in a number of capillaries are averaged. This fact is well seen in the accompanying tables (tables 2, 3 and 4) dealing with the diurnal variations in capillary pressure. So far as our present observations go, therefore, there is practically no variation in the

TABLE 2

*Determination of the pressure in a single capillary at different periods of the day*

10:30 a.m. ....	15.00 mm. Hg. (Readings taken by Dr. H.)
1:00 p.m. ....	15.00 mm. Hg. (Readings taken by C. S. D.)
Lunch	
2:00 p.m. ....	14.16 mm. Hg. (Readings taken by C. S. D.)
2:45 p.m. ....	14.50 mm. Hg. (Readings taken by C. S. D.)
3:00 p.m. ....	15.17 mm. Hg. (Readings taken by C. S. D.)

TABLE 3

*Determination of the pressure in four individual capillaries made on two days*

DATE	TIME	CAPILLARY A	CAPILLARY B	CAPILLARY C	CAPILLARY D
December 6. ....	10:30 a.m.	27	27	29	
	9:15 a.m.	23	23	22	
	11:15 a.m.		24	23	22
	12:30 p.m.		25	24	22
December 7. ....	2:00 p.m.			26	23
	3:30 p.m.			24	24
	4:30 p.m.	24		24	24
	5:00 p.m.	24			22

average values for a given group of capillaries at different times of day and on different days.

*The effect of intrathoracic pressure on capillary pressure.* Disturbances of intrathoracic pressure affect both the arterial (37) and venous (38) blood pressure. We have performed one experiment to study its effect on the capillary pressure. Our results, given in figure 8, show that forced expiration with the glottis closed (Valsalva experiment) causes a rise, while forced inspiration with the glottis closed (Müller experiment) causes a fall in capillary pressure. The curves are practically mirror pictures of one another. Since a decreased intrathoracic

TABLE 4

*Determination of the pressure in a group of capillaries studied on two consecutive days. Note the constancy of the pressure*

DATE		Hg. PRESSURE	AVERAGE PRESSURE
		mm. Hg.	mm. Hg.
November 5	a. m. ....	Ring finger	26½
		Small finger	24
	p. m. ....	Middle finger	23
November 6	a. m. ....	Ring finger	27
	p. m. ....	Small finger	24

The pressure in the capillaries of the ring finger is practically constant for the two days; likewise the pressure in the small finger capillaries.

negative pressure lowers the arterial and raises the venous and capillary pressures, while an increased intrathoracic negative pressure produces the opposite circulatory conditions, it follows that capillary blood pressure is more closely dependent upon venous than it is upon arterial pressure.

von Basch (39), who reports results similar to the above, has also emphasized the association of capillary with venous pressure. With these observations in mind, a systematic study of capillary pressure in cases of cardiac insufficiency which determines a high venous pressure and in cases of emphysema, asthma, pleural effusions, etc., which determine disturbances of intrathoracic pressure, should prove fruitful.

*The effect of venous compression on capillary pressure.* A rubber band was applied around the little finger and the pressure in one and the same capillary was taken with varying degrees of compression. Very marked compression which closes both the arteries and veins reduces the capillary pressure to zero. Moderate compression, however, (which compresses the veins only) raises capillary pressure (18 mm. Hg.); when the rubber band is removed the pressure returns to normal.

Normal.....	15.0 mm. Hg.
Rubber band firmly applied.....	0
Rubber band loosely applied.....	18.0 mm. Hg.
Rubber band removed.....	15.5 mm. Hg.

Hence we see that increased venous pressure (produced by compression) increases the pressure in the capillaries. The effects of venous compression were previously studied by von Basch and later by Krauss. Both of these observers concluded that a high capillary pressure resulted from the compression of its veins. von Basch even went as far as to formulate an hypothesis which was to the effect that capillary pressure was an index of the amount of venous stasis.

#### SUMMARY

A method for the study of capillary blood pressure is presented which differs from all of the previous methods in the criterion at which the readings are taken. It depends on the production of stagnation of the flow of corpuscles in the capillaries.

With this method capillary pressure in man can be determined in the fingers and in the toes. For the study of capillary pressure in animals (cat, dog, rabbit) the shaved ear should be used. Our apparatus readily adapts itself for such study.

We have studied the capillary blood pressure in normal individuals (sitting posture). The average pressure was 22.2 mm. Hg.

The study on the effect of temperature showed that cold lowers and heat raises capillary blood pressure.

Posture was found to vary capillary pressure although the hand was at heart level at all times. It was lowest in the recumbent, highest in the standing and midway between in the sitting posture.

The diurnal variation, contrary to previous opinions, is very slight.

Increased intrathoracic pressure raises capillary pressure. Diminished intrathoracic pressure lowers capillary pressure.

Venous compression causes an increased capillary blood pressure.

We believe that the prevalent conception that the redness of the skin is due to its capillaries and that pallor means capillary emptying, is erroneous. While undoubtedly contributing somewhat to the color of the skin, nevertheless the rôle of the capillaries is rather slight. The venous plexuses principally contribute to the color of the skin. The collapse of these plexuses is the principal cause of skin pallor resulting from compression.

The simplicity and the accuracy of our method gives us hope that it may find a place in the laboratory and in the clinic.

We propose as the name of our apparatus, the "micro-capillary tonometer."<sup>1</sup>

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## A PLETHYSMOGRAPHIC STUDY OF SHOCK AND STAMMERING IN A TREPHINED STAMMERER

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The experimental work here reported was done in January, 1920. The purpose of the experiment was to test the organic reactions accompanying stammering, with special reference to Dr. C. S. Bluemel's cerebral congestion theory of stammering, and to determine whether stammering, like shock, is accompanied by congestion in the brain, and hence by increased intracranial pressure, as was conjectured by me in the theoretical conclusions to "A plethysmographic study of shock and stammering" published in the April, 1919, number of this Journal.

*Subject.* The subject who took part in this experiment was born in Telsburg, Norway, November 14, 1875, and came to America in 1893. He went to school for nine years in Norway. He speaks Norwegian, Swedish and Danish as well as English, and used to speak Spanish; he stammers equally in all languages.

He is a laborer of low intelligence; he was a sailor for five years, a rigger for ten years, a pipe fitter for four years and a pile driver for two years. He attained a total score of 21, rating D, in Group Examination Alpha used in the United States Army, corresponding to a mental age of eleven years. His verbal imagery was the least vivid of any of the subjects tested in (5): his auditory was 1.03 compared with the average, 2.2; his kinesthetic was but 0.13 compared with the average, 0.8; and his visual but 0.27 compared with the average, for stammerers, 1.2. All types of his non-verbal imagery were vivid except the kinesthetic.

The subject spoke without hesitancy until, at the age of eight, he was so terrified on the ice by a boy who impersonated a bear that he was rendered speechless for two hours and has stammered ever since.

<sup>1</sup> The subject's salary and expenses were paid from a grant of \$100 from the Committee on Grants of the American Association for the Advancement of Science.



His stammering was increased at the age of thirty when he was shot by a robber, the bullet splintering the skull bone over the right eye and necessitating the trephine described by Doctor Cobb. The skin of the noticeable dip or hollow covering the trephine was loose and free in its movements and the pulsations were often conspicuous.

As far as can be learned, the subject inherited no tendency to stammer. He sings normally and reads and speaks without hesitancy when alone. He frequently stammers on words beginning with H, L and R and sometimes repeats the first letters of certain words without contortions, but often avoids this stammering by using synonyms for the words he wishes to speak. He is not nervous or excitable, and is not apparently embarrassed or sensitive about his stammering. Pneumograms traced while the kymograph was running at high speed showed that he breathes correctly before he speaks, but continues to speak after his lungs are empty. In short, the physical aspect of his stammering is far more prominent than the mental aspect, and the impediment in his speech, which is of a common type, would not be difficult to correct in a younger man who was willing to work conscientiously.

The subject was examined at the Massachusetts General Hospital by Dr. Stanley Cobb on January 8, 1920. His report follows:

*Complaint:* Stammering.

*Past History:* Smallpox at 21. Gonorrhea at 23 and 24. At about this time he also had a hard chancre for which he was treated with pills and inunctions for three months. He does not know of any other sickness except that of August 30, 1905, when he received a gunshot wound in the right forehead which perforated the skull. He was taken to the Long Island College Hospital and operated on. Evidently the bullet was removed and a small trephine hole left open.

*Physical Examination:* Cranial nerves: Smell normal. Ocular movements are normal. No squint or difficulty in convergence. Muscles of mastication strong and equal. The sensation on the face is normal. There is no facial weakness. Hearing tests show slight deafness in the left ear. The Rinne test if referred to the left. The defect seems to be in the air conduction apparatus. The drum appears normal. Taste is normal. The pulse is regular and slow (see special report in experiments). No weakness of the sternomastoid or trapezius muscles. Tongue protrudes in midline. The fundi show normal vessels. In the right eye the disk is sharp but in the left the margins are slightly hazy. Visual acuity is within normal limits. He does not wear glasses.

Reflexes: Biceps, triceps, knee and ankle jerks are all equal and if anything slightly depressed. The superficial reflexes of the abdomen and scrotum are slightly more active than usual and equal on the two sides.

Motor system: The muscle groups are strong and symmetrical. There is

no disturbance of gait, no ataxia, asynergia or aphonia. There is a slight fine tremor of the extended fingers. Rhomberg test normal.

**Sensory system:** There is no disturbance of touch, pain or discrimination on any part of the body.

**Endocrin system:** Thyroid not enlarged. Bony development normal. The distribution of the hair on the body and face is normal. The testicles are in normal position and well developed.

**Sympathetic system:** The pupils react to light and accommodation. They are round and equal. Skin reactions are slight. There is no dermatographia.

**Skull:** In the right fronto-temporal region 5 cm. from the midline is a defect in the bone irregularly circular in shape, its widest diameter being 2.5 cm. and its narrowest 2 cm. This pulsates visibly and when the patient leans over it is seen to bulge slightly.

**Respiratory system:** Lungs are clear. The nose, throat and tonsils are negative.

**Cardiovascular system:** The heart is not enlarged and the sounds are clear and without murmurs. The peripheral arteries are palpable but not thickened. The pulse is full. Blood pressure 110/80 (see special experiments). The aortic second sound is exaggerated and somewhat louder than the pulmonic second.

**Alimentary system:** The teeth are in fairly good condition except for three bad roots. The abdomen is level, soft and tympanitic. There are no masses felt.

**Blood:** No anemia. Wassermann reaction negative.

**Mental status:** General behavior: Normal, quiet and coöperative.

Stream of talk is slow but pertinent.

**Mood:** No depression or elation of spirits.

**Special preoccupations:** No worries, imaginations, delusions or hallucinations.

**Orientation:** Accurate for time, place and person.

**Memory:** Accurate for remote and recent events.

**General Information:** He knows the dates, names of the principal government officials, etc., but shows no interest in the affairs of the country.

**Speech:** There is a marked stammering, especially when embarrassed by the presence of a stranger. The sticking mainly occurs on the hard consonants. There is no aphasia, apraxia or astereognosis.

**Diagnosis:** An individual of the mentally dull type, probably not to be classed as a real defective, who has sustained a bullet wound of the right frontal region. There is no evidence of any brain injury.

**Apparatus.** The apparatus used in this experiment included the same Zimmerman kymograph, Sumner pneumograph, finger plethysmograph, piston recorder and the two electromagnets described in (6), pages 293 to 302, a less sensitive tambour than the one used in my earlier work, a second piston recorder like the first, and two forms of brain plethysmograph.

The first form of brain plethysmograph, which I will call the rubber plethysmograph, consisted of a hard rubber cup having an edge of soft rubber. A metal tube led out through the top side of the cup, and a rubber tube connected this with a syringe and piston recorder as in my earlier experiment. This plethysmograph was held in place by a single bandage passed around the subject's head from front to back. As the pressure of this bandage gave the subject a severe headache, most of the experiments were performed with the second form of brain plethysmograph, consisting of a glass funnel 4.7 cm. in inside diameter, which was cemented to the subject's forehead with collodion and removed with ether. This glass plethysmograph had two other advantages over the rubber one: it was not pressed against the forehead by bandages whose pressure varied with head movements, and an observer could detect sudden pulsations within the trephine which registered on the drum so much like movements, that in the records where the rubber plethysmograph was used I mistook them for movements and called in expert witnesses while the records were being traced to affirm that these abrupt rises were not due to movement in the large majority of cases.

With the apparatus described in (6), page 300, it was found that a rise of 1 mm. on the records always denoted an increase in volume of the brain of 3.0 cu. mm. when the rubber plethysmograph was used, and of 2.5 cu. mm. when the glass plethysmograph was used.

*Procedure.* The procedure and arrangement of apparatus was practically the same as described in (6), pages 302 to 306, except that the kymograph was run at higher speed to show the pulse. As five tracings were being made at the same time, it was very difficult to readjust one writing needle without throwing another out of adjustment. Being most interested in the brain tracing, I neglected the other writing needles when the one tracing the brain volume needed attention. For this reason, the needle which traced the finger volume and which moved freely only when it just touched the drum was frequently pressed too tightly against the drum to make a true tracing. The vasomotor reactions studied in (6), where this delicate pressure was kept constant, are far more reliable than those in this study.

As the pressure of the needle tracing the finger plethysmograms could not be kept uniform, no attempt was made to measure the finger plethysmograms; these were recorded simply as increases or decreases.

To determine what route the writing needle connected with the brain plethysmograph would have taken had the subject done no mental or

physical work and been given no stimulus, I placed the celluloid triangle along the crests of the highest pulses in the troughs of the Traube-Hering waves of the rest period immediately preceding and of that immediately following the period of work or disturbance. As the Traube-Hering waves were never large in these rest periods, and as the pulses at their troughs were much more uniform than those at their crests, this gave a fairly accurate reference line, though of course measurements made from it were necessarily approximate compared with those made from as accurate a reference line as was determined for the finger plethysmograms in (6). The lowest point on each plethysmogram, like the highest, was always considered to be the crest of a pulse at any phase of a Traube-Hering wave.

About half of the curves had to be discarded because of very slight leaks which developed in the brain plethysmograph when movements made by the subject loosened the bandage of the rubber plethysmograph or weakened some spot in the collodion which held on the glass plethysmograph; the discarded curves confirmed those retained as to rises and falls, but did not admit of accurate measurement. If the brain plethysmogram was reliable, the curve was retained, no matter how imperfect the finger plethysmogram.

The subject was seated in a large Morris chair so inclined that the trephine was nearly horizontal when he laid his head back in the chair; this put the least strain on the collodion which held on the glass plethysmograph.

An observer, seated close to the subject in most of these experiments, pressed a key whenever the subject moved his head, thereby enabling me to determine which abrupt rises in the brain plethysmograms were due to a rapid change in the brain's volume. In experiments arranged to study the effect of movements, it was found that small movements showed little if at all in the records, whereas big or quick movements were noticeable, raising the head or turning it to the right appearing to give a rise, lowering the head or turning it to the left appearing to give a fall. Curves containing head movements after which the writing needle did not return to normal were discarded.

Table 1 contains a brief summary of my results. The percentages of increases, decreases, no changes and complex reactions are given for both brain and finger volume: + denotes increase in volume; - denotes decrease in volume; 0 denotes no change in volume; +(-) denotes a prolonged increase followed by a short decrease; and so on. Rise in millimeters was measured to the highest point of the brain

plethysmogram from the path its writing needle would have traced had the subject's mind been a blank. This normal path was assumed to pass through the crest of the highest pulse at the trough of each Traube-Hering wave. The column entitled "Increase in height of brain pulse" gives the number of times greater in height the average pulse during the performance of an assigned task was than the normal pulse in the rest periods immediately preceding and immediately following the period of activity. The maximum pulse referred to in the next column is the highest brain pulse traced during the period of activity.  $t$  gives the time in seconds from the beginning of an assigned task to the time when the recording needle connected with the brain plethysmograph first attained its maximum rise.  $T$  gives the time in seconds from this maximum rise to the time when this recording needle first returned to normal, that is, to the path it would have taken had the subject's mind remained a blank. Or, if the time of maximum rise occurred while the subject was performing an assigned task,  $T$  was measured from the instant of completion of the task instead of from the end of  $t$ .

Every period of stammering while reading or speaking showed marked increase in brain volume usually accompanied by a greatly increased pulse for at least part of the period (see figs. 4, 5 and 6). Six periods of reading, averaging 77 seconds in length, gave rises of from 23 to 88 mm., averaging 49 mm. The maximum height of pulse registered during each reading period ranged from 1.7 to 5.0 times the normal size of pulse before and after the period of stammering, averaging 3.3 times the normal pulse. Twelve periods of talking, averaging 95 seconds in length, gave rises of from 32 to 70 mm., averaging 53 mm., and maximum pulse of from 1.4 to 7.0 times the size of the normal pulse, averaging 4.2 times the normal pulse. Thus there appeared to be slightly greater cerebral congestion during talking than during reading. The rise for these reading and talking periods, taken together, ranged from 23 to 88 mm., averaging 52 mm.; and the maximum pulse for the same periods ranged from 1.4 to 7.0 times the size of the normal pulse, averaging 3.9 times the normal pulse.

The maximum rise occurred on the average near the middle of the period in both reading and speaking. The brain volume and pulse became normal in from 20 to 46 seconds, averaging 33 seconds, after the end of the reading periods, and in from 0 to 82 seconds, averaging 37 seconds, after the end of the talking periods.

TABLE 1  
*Vasomotor changes accompanying various physical and mental states*

WHAT SUBJECT WAS DOING	NUM- BER OF CURVES	FINGER VOLUME	BRAIN VOLUME	BRAIN VOLUME RISE IN MILLIMETERS			INCREASE IN HEIGHT OF BRAIN PULSE			NUMBER OF TIMES MAXIMUM PULSE EXCEEDED NORMAL			t	T
				Maxi- mum	Aver- age	Mini- mum	Maxi- mum	Aver- age	Mini- mum	Maxi- mum	Aver- age	Mini- mum		
		per cent	per cent											
Stammering.....	18	0 62 + 12 - 6 +- 19	+	23	52	88	Irregular			1.4	3.9	7.0	Middle	35
Reading aloud with no one in the room.....	1	+	+	53	53	53	1.8	1.8	1.8	2.5	2.5	2.5	Middle	18
Reading normally.....	6	0 67 + 33 - 17 0 17	+	-6	9	20	1.5	?	4.0	1.0	3.3	8.0	End	32
Silent reading.....	7	0 57 + 29 +- 14 0 29	+	0	14	29	1.0	1.0	1.0	1.2	1.4	2.0	Middle	55
Other mental work.....	8	+ 50 0 33 +- 17 0 13	+	0	19	40	-?	+	3.0	1.2	2.5	4.0	End	55
Physical work.....	6	+	+	19	21	25	2.4	5.4	8.0	2.8	7.5	12.0	Varied	54

Increased intrathoracic pressure.....	5 { + 20 0 40 - 40	+ (-)	52	69	82	+	+	+	1.5	11.3	22.5	Start	36
Clearing throat.....	2	+ (-)	63	66	70	+	+	+	1.6	1.7	1.8	Start	16
Sniffing.....	2	+	58	59	60	2.0	4.0	6.0	3.0	6.5	10.0	End	36
Deep breathing.....	5 { 0 67 - 33	+ 20 - 80	5	-12	-32	-1.7	-2.3	-3.0	1.0	3.3	10.0	End	43
Fear of stammering....	4	+	15	28	45	+	+	4.0	2.1	4.5	5.8	?	?
Shock.....	10 { 0 87 - 13	+ 80 0 20	0	16	38	+	+	+	1.0	3.8	8.3	15	23

The finger plethysmograms gave about an equal number of vasodilatations and vasoconstrictions in both reading and speaking, two +, one -, one +-, one -+, one -+-, and ten no change; finger movements made it impossible to determine the other reactions.

It is important to determine what part of the increased intracranial pressure is due to stammering and what is due to the ordinary mental and physical work of reading or speaking. The subject read aloud in the room by himself without hesitancy; but there were the usual disturbances in breathing and the employment of superfluous effort which are usual in stammering whether reading alone or in public; and the single record (see fig. 3) in which the results were not injured by movement or by the needles' leaving the drum when no experimenter was in the room gave a maximum rise of 53 mm. in the middle of the 140 second reading period, an average pulse 1.8 times the normal pulse before and after reading, and a maximum pulse 2.5 times the normal, 25 mm. compared with 10 mm. The volume and pulse returned to normal more quickly, however, than after a period of severe stammering, this being accomplished in about 18 seconds. The finger plethysmograms showed marked vasodilatation. This single record indicates that a stammerer may have increased intracranial pressure while reading aloud or speaking whether he is stammering or not, but that this intracranial pressure is highest during periods of severe stammering.

A more satisfactory comparison would be that of the subject reading aloud a given passage and reading aloud another from the same book after he was cured. I was, fortunately, able to teach him in five days to read without hesitancy to any person who came into the room, and believe a comparison of records obtained while he was thus reading normally with the above stammering reading records will be a fair comparison of reactions to stammering and normal speech. He read aloud in this way in six periods free from movements in periods averaging 65 seconds in length (see fig. 7). Every time the subject breathed he raised his head, causing a distinct rise on the brain plethysmogram. A wave was thus caused in the curve which might be termed an indirect breathing curve. This curve was neglected in making measurements of changes in brain volume. One record gave no change in brain volume, one gave -6, + to 0, -6, and four gave rises from 12 to 20 mm., averaging 15 mm. The average pulse during these periods varied greatly and could be determined in only two records, in one increasing from 5 to 20 mm. and in the other decreasing from 12 to 8 mm. In two of the records the maximum pulse did not increase at all,



and in another it was as high as 8 times the normal, averaging 3.3 times the normal pulse. The greatest rise occurred in the middle of the period having the greatest average pulse and near the end of the other 5 periods. The time of recovery was 24 seconds in one, 40 seconds in another, and in doubt in 4. The finger plethysmograms showed a rise in two and no change in four of the records. There appears, therefore, to be much greater intracranial pressure in stammering reading than in normal reading, the average rise being 49 mm. compared with 15 mm.

To determine how much of the rise in normal reading was due to the mental work of the reading itself, I had the subject read silently seven passages in periods averaging 46 seconds (see fig. 2). The brain tracing showed no change in two,  $+-$  in two, and  $+$  in three; where there was a  $+$  in any part of a reaction, it ranged from 12 to 29 mm., averaging 20 mm., and will therefore account for the rise in normal reading aloud. The average pulse was about the same as the normal pulse, and the maximum pulse ranged from 1.2 to 2.0, averaging 1.4 times the normal. Hence the mental work of reading does not account for the increased pulse during normal reading; on the other hand, it will be seen that the physical work does account for this. The greatest rise occurred near the middle of the reading period and the time of recovery averaged 55 seconds, as it did in the case of other kinds of mental work. The finger plethysmograms showed one  $+$ , one  $+-$ , and four no changes.

Other kinds of mental work, including checking additions, multiplications and divisions (see fig. 2), and counting the number of E's on a page, gave reactions similar to silent reading in eight 1 to 3 minute periods. The brain volume remained the same in one,  $0+$  in another,  $(+)(-)+$  in another,  $-+$  in another, and  $+$  in four, the rise varying from 0 to 40 mm. and averaging 19 mm. The average pulse during periods of mental work was the same as the normal in four tracings, was lower in one, and was higher in three, as much as 3 times higher in one case; the maximum pulse was from 1.2 to 4.0, averaging 2.5 times the normal, being greater than that in reading silently. The maximum rise occurred near the end of the period of mental work, and the time of recovery ranged from 15 to 99 seconds, averaging 55 seconds. The finger plethysmograms showed no change in two curves,  $+$  in three,  $+-$  in one; and two were in doubt.

Periods of physical work on the ergograph used by Anderson and described in (1) page 42, gave six clear cut results (see fig. 1). There was

one fatigue period which lasted 184 seconds; the other test periods lasted one minute each. There was a rise of the brain plethysmogram with increased pulse in five periods, (+)(-)0 with larger pulse in the sixth. The rises ranged from 19 to 25 mm., averaging 21 mm., including the increased pulse which averaged 16 mm., about half of which is above the line drawn through the center of the pulse. The average pulse during physical work was from 2.4 to 8.0 times the normal, averaging 5.4 times the normal, and remained nearly uniform throughout the period of work, the maximum ranging from 1.2 to 1.7 and averaging 1.4 times the average pulse during the period of work. In the case of the long fatigue period the maximum rise occurred near the end; and in the short periods its position varied. The time of recovery ranged from 18 to 72 seconds, averaging 54 seconds. The finger plethysmograms gave + in five of the curves and (-)+ in the other: in this one the brief fall was due, no doubt, to the increased mental activity due to the change of task. Physical work, therefore, is accompanied by slight increase in both brain and finger volume, and by marked increase in brain pulse.

It seemed well, also, to study the brain volume during increased intrathoracic pressure, for this occurs with the muscular spasms which accompany stammering. The subject was asked, therefore, to clear his throat and also to bear down as if straining at the stool. In both cases the immediate rise was so great and so abrupt that I thought it was caused by a movement of the head until I saw through the glass plethysmograph that the skin over the trephine flattened out instantly at these times and that the subject did not move. Both showed a brief fall in brain volume after a marked rise, then a slow return to normal, the high pulse gradually decreasing (see fig. 9). The rise varied from 52 to 82 mm., averaging 68 mm. The maximum pulse averaged 1.7 times the normal in the clearing of the throat, and 11.5 times the normal in the bearing down, being 23 times the normal in one case, and even higher in discarded records where the piston of the recorder came out of its barrel. The time of recovery averaged 16 seconds for the clearing of the throat, and 36 seconds for the bearing down.

In one record chewing chocolate gave a rise in brain volume of 64 mm., the average pulse during chewing increasing from the normal of 9 to 20, and the maximum pulse to 45. This rise was not due to the pleasant sensation of the chocolate, as there was no appreciable rise during the half-minute he held this on his tongue before chewing; or

to the opening and closing of the mouth per se, as this gave a fall of 7 mm. and a low pulse when the subject opened his mouth every time he inhaled and closed it every time he exhaled. It must have been due to the physical work of chewing, and might be expected in any stammerer who forces words with his jaws.

A comparison of different kinds of breathing was made to learn what effect these had upon intracranial volume. Holding a deep breath caused a fall at first, then a rise until the subject breathed. Twelve seconds after the subject had kept his lungs empty for fourteen seconds, a maximum rise of 38 mm. occurred with maximum pulse nearly double the normal. Two records of sniffing (see fig. 8) gave rises of 58 and 60 mm. with average pulse during the period 6 times the normal in the first and twice the normal in the second. The time of recovery was 40 seconds in the first and 33 seconds in the second. There was no change in finger volume. The actions of clearing the throat and sniffing both tend to fix the diaphragm and thus cause increase of intrathoracic pressure.

Deep breathing, on the other hand, had quite a different effect (see fig. 8). Three periods gave (+) —, one +, and one — in the brain tracing, and one — and two no change in the finger tracing (two were obscured by movement). The single instance where the change in brain volume was + throughout was but 5 mm.; the four decreases ranged from 10 mm. to 32 mm., averaging 16 mm. The average pulse during this deep breathing either remained the same or decreased, the decrease ranging from 1.7 to 3 times as low as that of normal, averaging 1.8 times as low. The greatest decrease occurred at the end of each period.

In one record I had the subject read aloud normally, then breathe deeply, then read normally again, and then read silently, without pausing between these periods. The curve showed little change for these different periods of mental and physical work. The volume remained about 12 mm. above normal throughout the first normal reading period, averaged 10 mm. during the period of deep breathing, rose from 10 to 13 mm. during the second period of normal reading, and kept at 12 mm. during the period of silent reading, the pulse being higher for the silent reading period than for the other periods.

Fear of stammering was also compared with shock as in my earlier study, but in spite of my many efforts to get the subject to live over again an experience in which he feared that he would stammer, I was able to cultivate this emotion only four times. On the most successful

occasion (see fig. 5), I told him that a lady to whom my pupils found it most difficult to speak would enter the room in a few minutes and ask him some questions; she entered at the psychological moment. The four above mentioned brain plethysmograms gave rises of 15, 19, 33, and 45 mm. with increases in pulse, the average pulse increasing 4 times above the normal in the 33 mm. rise in figure 5. No change in finger volume could be detected in any of these curves.

My subject's reactions to shock confirmed those of Shepard, (7) and (8), discounting the fact that my subject was less emotional (see fig. 10). There was no change in brain volume in two of the ten reactions to the quick stimulus of a single loud noise, and an increase in the other eight ranging from 9 to 38 mm., averaging 20 mm. The maximum pulse ranged from no increase to an increase of 8.3 times the normal pulse, averaging 3.8 times the normal pulse. The maximum rise occurred from 5 to 18 seconds after the stimulus (30 seconds in the case of one stimulus whose length was in doubt), averaging 15 seconds. The time of recovery ranged from 14 to 34 seconds, averaging 23 seconds. The finger plethysmograms showed a decrease in one case, no change in six cases, and movements in the other three; this proves my assertion that this subject was far less emotional than the subjects in my earlier experiments (6).

Now and then I noted a decided rise in a rest period and asked my subject to tell me at the end of the experiment whether he was thinking of something pleasant or unpleasant just then; I found a reason for every such rise. In one case something exciting flashed in his mind for 4 seconds causing a rise of 12 mm. A very pleasant emotion for which he reported he was miles away caused a rise of 23 mm. (see fig. 1) and a maximum pulse 13 times the normal; the finger volume also increased in this case. I was unsuccessful in getting him to cultivate emotions at will, so had to study emotions by retrospective reports in this way.

In one case I had an assistant make a loud noise behind the subject in the middle of a speaking period and found that this made him stammer worse and greatly increased the brain volume; this noise startled the subject so that he moved enough to make a reference line inaccurate, but not enough to spoil the record.

So far as I know, there has been no previous work on a trephined stammerer. A comparison of my results with those of Berger, Mosso, Shepard and Weber upon normal speakers, summarized under "brain volume" at the bottom of table 1 (6, p. 289), shows that my results agree in every case with those of Mosso and Shepard, and agree with

those of Berger and Weber in the reactions to stimuli and to mental and physical work.

A few readings of the subjects systolic and diastolic blood pressure were taken at intervals while the experiments were in progress. The subject's normal blood pressure averaged 110/80. When asked a sudden question and put completely off his guard, Doctor Cobb found the subject's blood pressure to be 145/108. Bearing down sent the blood pressure up to 125/104, slight stammering to 122/?, physical work on the ergograph to 118/?. Increase in brain volume would seem, therefore, to have a high correlation with increase in blood pressure.

#### SUMMARY

1. Pronounced shock, fear of stammering and emotions of every kind always brought about increase in brain volume accompanied by increased size of pulse.

2. Mental work was accompanied by slight congestion in the brain in a majority of cases with little if any increase in pulse; physical work was accompanied by slightly more congestion in the brain with marked increase in size of pulse. Physical work was accompanied by slight vasodilatation in the finger; mental work, including silent reading, by vasodilatation in the finger in 38 per cent of the curves, by vasodilatation followed by vasoconstriction in 16 per cent, and by no change in 46 per cent.

3. Sniffing was accompanied by marked increase in brain volume with increased size of pulse; deep breathing by decrease with slightly decreased size of pulse.

4. Change of task was accompanied by increase in brain volume with increased pulse and was responsible for many temporary rises.

5. Normal reading aloud was accompanied by slightly less increase in brain volume than was silent reading.

6. Stammering was accompanied by much more marked increase in brain volume than could be accounted for by either the physical or mental work used in normal speech.

7. When the stammerer read as normal speakers do, there was a return of the brain volume to normal; it is reasonable to conclude that increase in brain volume is an important factor in the production of stammering.

8. In order to correct stammering, both the fear of stammering and the abnormal muscular contractions which usually accompany stammering must be eliminated.

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(This has not been published, but may be consulted in the Widener Library of Harvard University.)
- (2) BERGER: Über die Körperlichen Ausserungen psychischer Zustände, Jena, 1904-7.
- (3) BLUEMEL: Stammering and cognate defects of speech, New York, 1913.
- (4) MOSO: Über den Kreislauf d. Blutes im mensch. Gehirn, 1881.
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- (6) ROBBINS: This Journal, 1919, xlviii, 285.
- (7) SHEPARD: Amer. Journ. Psychol., 1906, xvii, 522.
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## EXPLANATION OF FIGURES

The top line in all figures is the time line, the second line is the stimulus line, the third line is the thoracic pneumogram, the fourth line is the finger plethysmogram and the bottom line is the brain plethysmogram.

Each notch in the time line represents two seconds unless otherwise stated.

A notch on the stimulus line indicates when a stimulus was given or when the subject began or stopped reading or speaking, and will be explained in the description of each record.

The top of the pneumogram indicates empty lungs and the bottom full lungs, just the reverse of the notation in my former monograph (6).

The top of each plethysmogram indicates vasodilatation, the bottom vasoconstriction.

The following curves are all typical reactions; I have avoided reproducing extremes or abnormal curves. If any reader wishes to see my other curves, he is invited to examine those in my album at the Boston Stammerers' Institute.

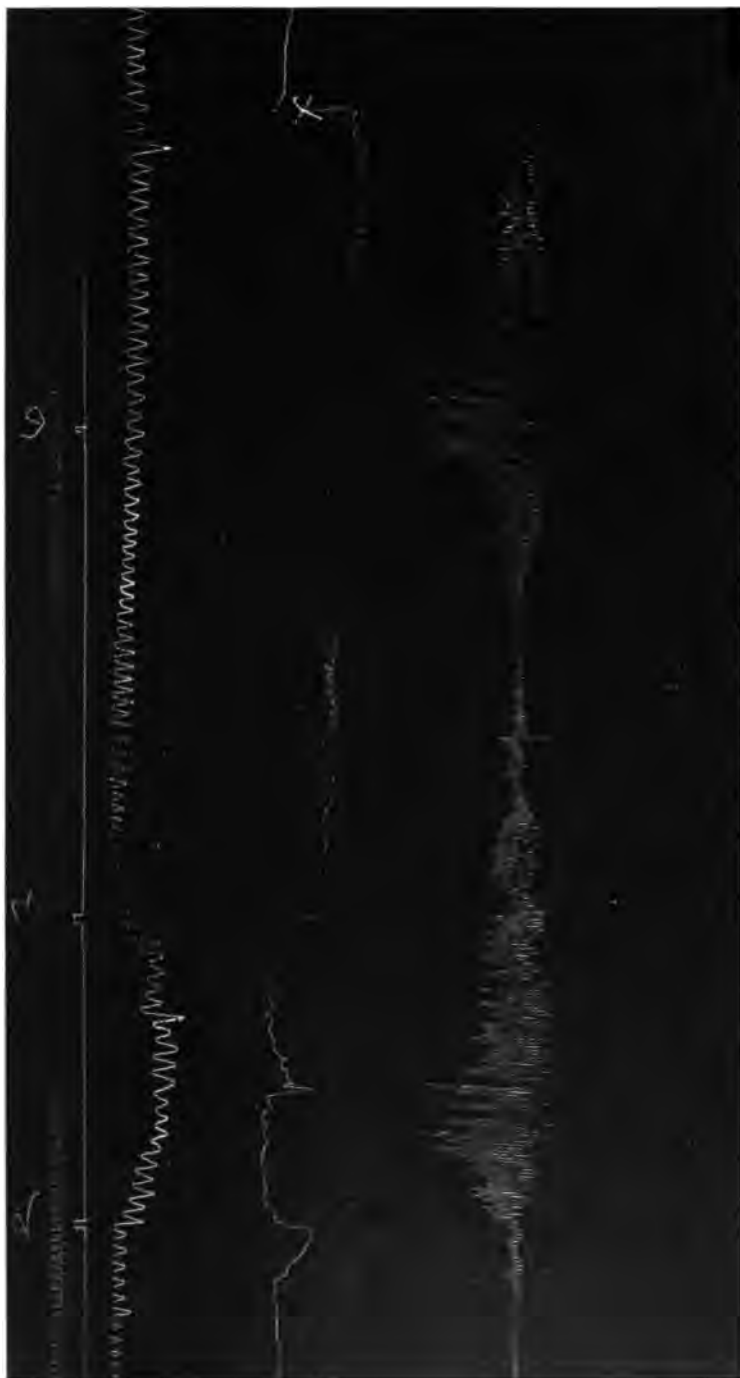


Fig. 1 shows the changes in the brain pulse during a one-minute period of physical work on the ergograph. At the notch marked 3 the subject began to work and at 4 he stopped working. At 5 he was asked to remember what he was then thinking of; he reported at the end of the experiment that his mind was way off and that he was thinking of something very pleasant. Note that the height of the brain pulse increased during both the period of physical work and the pleasant emotion, and that the brain volume remained about the same. The finger volume increased in both cases, movement making the amount of the rise uncertain in the period of physical work. Three-fifths natural size.



Fig. 2 shows the changes in brain volume accompanying mental work. From notch 14 to notch 3 (the left hand 3) the subject checked up a long division; from 3 to 16 he relaxed. At 15 my assistant accidentally dropped a small object. At 16 the subject picked up a book and read silently to 3 (the right hand 3), where he again relaxed. At the notches marked *M* my assistant saw the subject move his head slightly. Note that the brain volume increased with little change in pulse during both periods of mental work; the brief initial rise in these two periods was probably caused by the marked changes in breathing recorded in the pneumogram. There was little if any change in finger volume. One-half natural size.





Fig. 3 shows the changes in brain volume and pulse while the subject read aloud in a room by himself without stammering before taking lessons. At 12 the experimenters left the room and the subject began to read; at 13 they returned and he stopped reading. Compare this figure with the next three and note that the brain volume increased as much during this period of reading in a room by himself as it did during some periods of stammering. One-half natural size.



Fig. 4 shows the changes in brain volume during a long period of severe stammering while reading. At 8 the subject began to read aloud and at 9 he stopped reading. At 10 the subject yawned and at 12 I spoke to him. Note the marked rise in brain volume which accompanied stammering; the change in pressure of the writing needle on the drum occasioned by this big rise decreased the excursion of the needle on the drum and thus made a comparison of the pulse impossible. One-half natural size.



Fig. 5 shows the changes in brain volume and brain pulse accompanying fear of stammering. At 2 the subject was informed that a lady, to whom all stammerers found it very difficult to speak, would enter the room in about fifteen seconds and ask him some questions. She opened the door just before 3 and asked him brief questions from 3 to 4 which he answered briefly, stammering slightly. At 4 he relaxed. Note that both the brain volume and the height of brain pulse increased nearly as much during fear of stammering as during the actual stammering. One-third natural size.

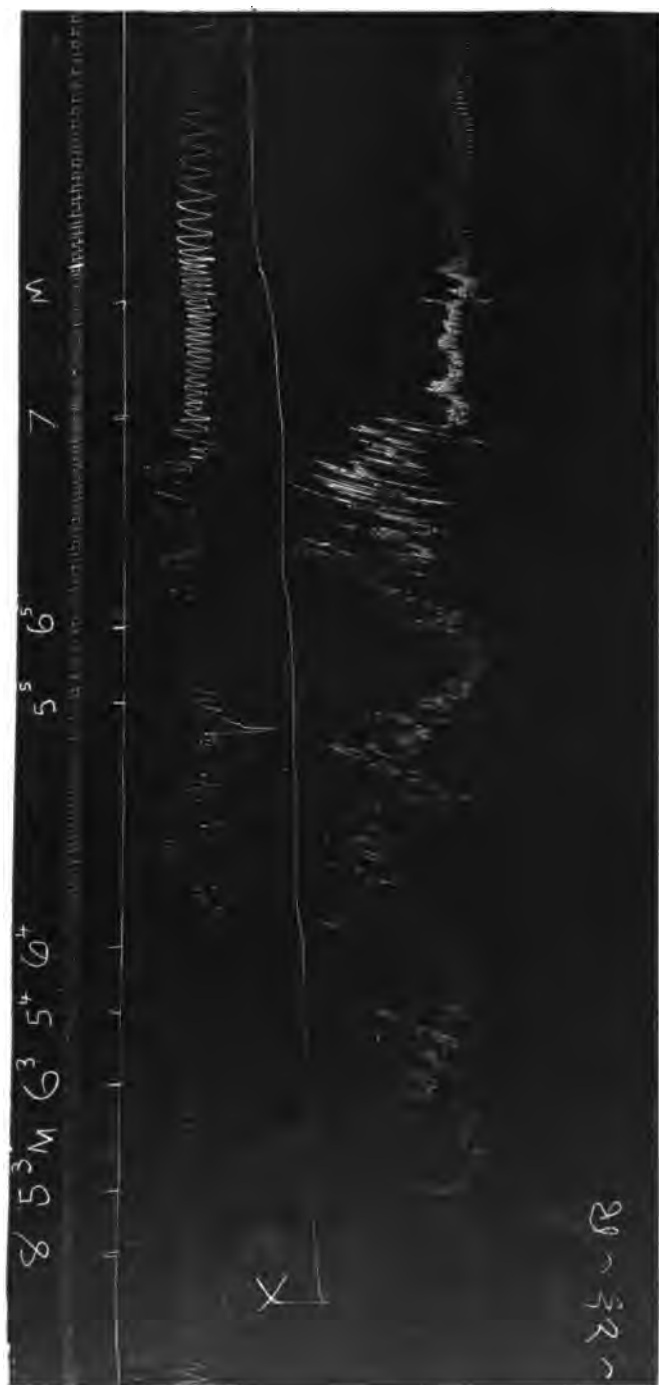


Fig. 6 shows the changes in brain volume and brain pulse during stammering speech. At 8 the subject was told that a Harvard instructor would ask him some questions in about 15 seconds. At 5 this instructor asked the subject an unexpected question which the subject answered at 6. The subject got much warmed up while answering question five and stammered more severely than usual in answer 6. At 7 the subject relaxed, and at M he moved his head slightly. Note the increase in both brain volume and pulse during the periods of stammering. One-half natural size.

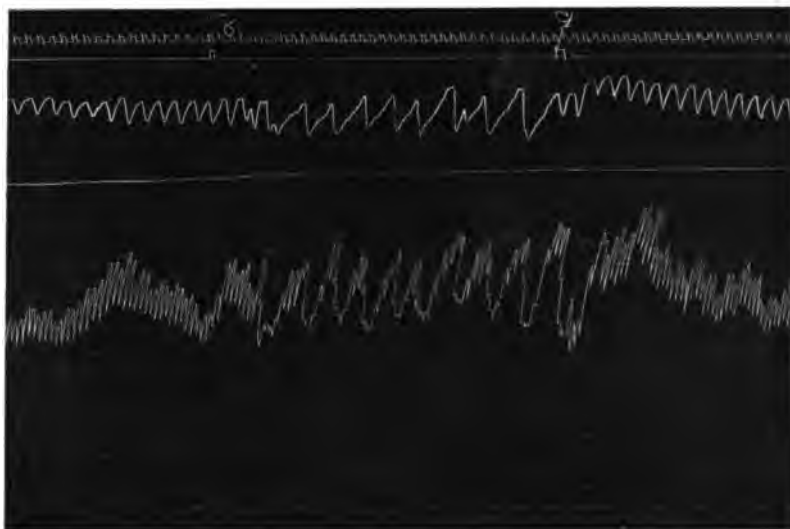


Fig. 7 contains a brain plethysmogram traced during a period of normal reading aloud after the subject had taken lessons. He read aloud normally from 8 to 9, and read silently from 9 to the end of the curve. Note that there was little change in brain or finger volume, and that there was a noticeable secondary breathing wave in the brain plethysmogram. Two-fifths natural size.



Fig. 8 shows the comparative changes in brain volume and pulse during slow deep breathing and sniffing. The subject breathed slowly and deeply from 2 to 4, relaxed from 4 to that point before 3 where the small waves in the large waves of the pneumogram show that he began to sniff. He sniffed as far as 5, and then relaxed until the end of the curve. At 5 the kymograph stuck for a few seconds. Note that both brain volume and brain pulse decreased slightly during the period of deep breathing and increased considerably during the period of sniffing. Note the same secondary breathing wave in the brain plethysmogram that was seen in figure 7. One-half natural size.



Fig. 9 shows the changes in brain volume and pulse accompanying abdominal contractions. At 9 the subject was asked to bear down and at 12 he was asked to clear his throat. The length of time the subject held his breath represents the number of seconds he bore down; he did not wait for the signal at 9. Note that the brain volume increased noticeably with both kinds of abdominal contractions. The initial abrupt rise under each 12 is not due to movement; one could see the skin over the trephine protrude every time the subject cleared his throat. Two-thirds natural size.

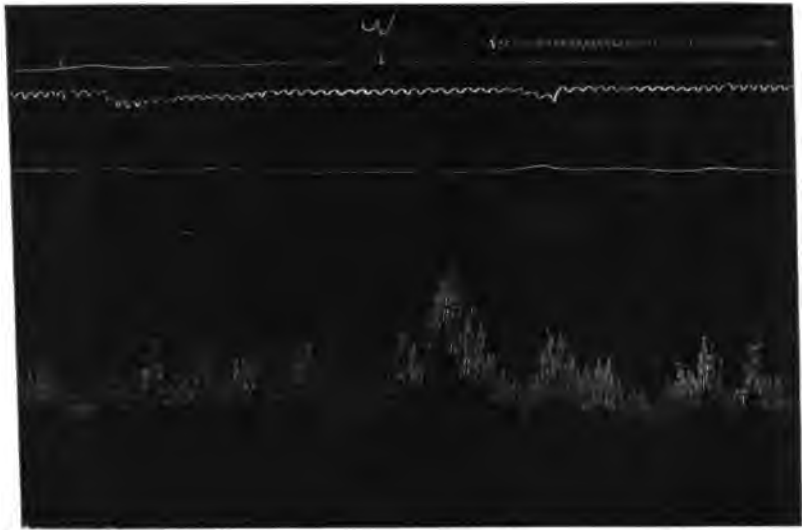


Fig. 10 shows the changes in brain volume accompanying shock. At *W* a shrill whistle was blown unexpectedly. The clock stopped running for a few minutes at the beginning of this curve. Note that the brain volume increased soon after the whistle was blown and that the finger volume remained constant. Two-fifths natural size.



# THE CHEMICAL CONSTITUTION OF ADENINE NUCLEOTIDE AND OF YEAST NUCLEIC ACID

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## I. A COMPARISON OF THE RATE AT WHICH PHOSPHORIC ACID IS SET FREE FROM YEAST NUCLEIC ACID WITH THE RATE AT WHICH PHOSPHORIC ACID IS SET FREE FROM THE INDIVIDUAL NUCLEOTIDES

After an extended study of the rate at which phosphoric acid is split from yeast nucleic acid by hydrolysis with mineral acid, Jones and Riley<sup>1</sup> concluded that the nucleic acid liberates its phosphoric acid by two widely different laws: and they predicted that if the individual nucleotides were ever prepared from yeast nucleic acid and examined in this respect, each of the purine nucleotides would be found to obey one of these laws and each of the pyrimidine nucleotides would obey the other. Various preparations of mixed nucleotides were afterwards obtained and their conduct justified this assumption since each nucleotide preparation was found to set free its phosphoric acid in accordance with the proportion of purine and pyrimidine material that it contained.<sup>2</sup> Finally Jones and Kennedy<sup>3</sup> found that the nucleotide groups are burned from yeast nucleic acid by potassium permanganate in a definite order, adenine nucleotide being the last to go; so that it was possible to obtain pure adenine nucleotide in this way.

The substance consists of characteristic long transparent needles of the composition  $C_{10}H_{14}N_5PO_7 \cdot H_2O$  and its possession furnished an excellent opportunity to ascertain the rate at which phosphoric acid is set free from a purine nucleotide. This rate was found to be the same as that of the previously determined rate for guanine nucleotide,<sup>4</sup>

<sup>1</sup> Journ. Biol. Chem., 1916, xxiv, i.

<sup>2</sup> Jones and Germann: Journ. Biol. Chem., 1916, xxv, 100. For the analytical data see Jones and Read, Journ. Biol. Chem., 1917, xxix, 123.

<sup>3</sup> Journ. Pharm., 1918, xii, 253; 1919, xiii, 45.

<sup>4</sup> Jones and Read: Journ. Biol. Chem., 1917, xxxi, 337.

both being the rate that Jones and Riley<sup>5</sup> had predicted for purine nucleotides.

This relation is expressed diagrammatically in figures 1 and 2, which are constructed with the same system of coördination and the same linear units. Abscissae represent time and ordinates represent weights of

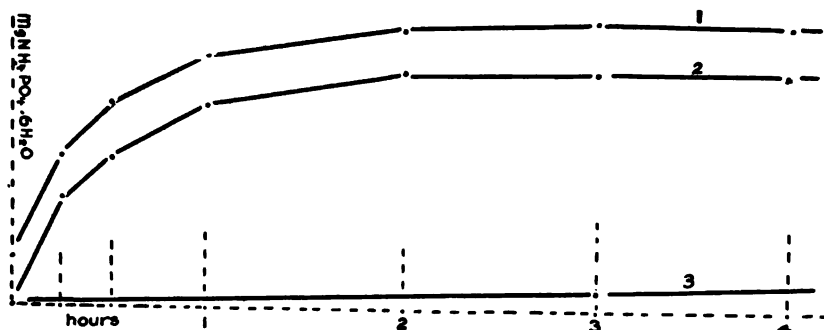


Fig. 1. The two curves at the top show the rate at which phosphoric acid is set free from the purine nucleotides. The straight line shows the rate for the pyrimidine nucleotides.

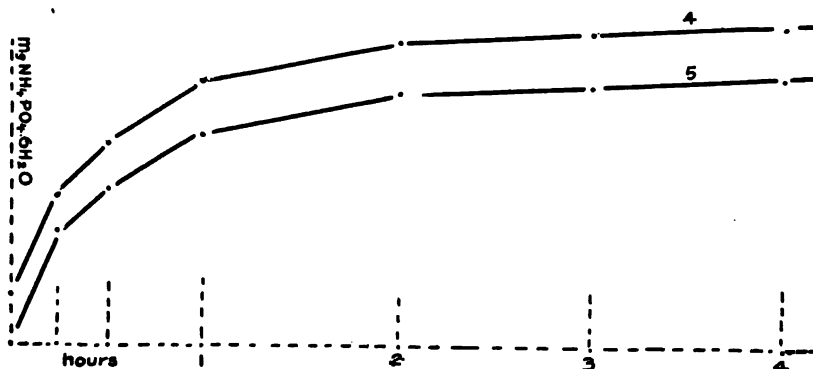


Fig. 2. The upper curve is a fusion of 3 with 1 or 2. The lower curve shows the rate at which phosphoric acid is set free from yeast nucleic acid.

phosphoric acid (expressed in terms of ammonium magnesium phosphate). In order to avoid confusion, the curves in each diagram have different origins placed vertically above one another.<sup>6</sup>

<sup>5</sup> Journ. Biol. Chem., 1916, xxiv, i.

<sup>6</sup> For analytical data see end of the article.

The upper curve of figure 1 was constructed from experimental data obtained with guanine nucleotide three years ago by Jones and Read.<sup>7</sup>

The lower curve of figure 1 was similarly constructed from experimental data obtained more recently by Jones and Kennedy<sup>8</sup> with adenine nucleotide.

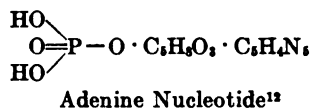
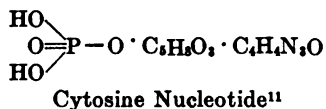
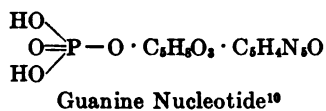
The two curves are practically identical and may be called the law for purine nucleotides.

The straight line of figure 1 was drawn from experimental data obtained by Jones and Read<sup>9</sup> with a mixture of the two pyrimidine nucleotides. It shows the very slow regular rate which may be called the law for pyrimidine nucleotides.

The upper curve of figure 2 was constructed by fusing the pyrimidine curve with either one of the purine curves of figure 1.

The lower curve of figure 2 was constructed from experimental data obtained with yeast nucleic acid, and it practically coincides with the lower curve.

This coincidence points to a very definite conclusion. If it be granted that the rates are expressions of phosphoric acid linkages, then the phosphoric acid linkage of yeast nucleic acid must coincide with the phosphoric acid linkages of its four component nucleotides.



<sup>7</sup> Journ. Biol. Chem., 1917, xxxi, 337.

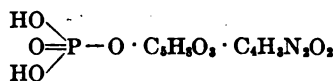
<sup>8</sup> Journ. Pharm., 1918, xii, 253; 1919, xiii, 45.

<sup>9</sup> Journ. Biol. Chem., 1917, xxxi, 39.

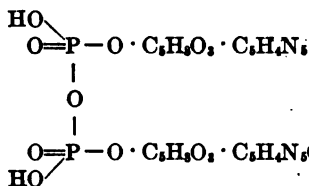
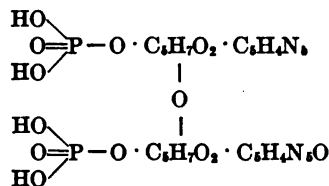
<sup>10</sup> Jones and Richards: Journ. Biol. Chem., 1914, xvii, 71.

<sup>11</sup> Thannhauser and Dorfmueller: Ber. d. d. chem. Gesellsch., 1918, li, 467. Zeitschr. f. physiol. Chem., 1919, ciii, 65.

<sup>12</sup> Jones and Kennedy: Journ. Pharm., 1918, xii, 253; xiii, 45.

Uracil Nucleotide<sup>13</sup>

If yeast nucleic acid is a chemical combination of the four nucleotides, then in this union the phosphoric acid groups of the nucleotides must not be disturbed. No additional phosphoric acid linkages can be introduced. The four nucleotides possess together eight replaceable hydrogen atoms; so also, yeast nucleic acid must contain eight replaceable hydrogen atoms. Therefore the nucleotide linkages of yeast nucleic acid cannot be through the phosphoric acid groups: the linkages cannot involve any one of the four phosphoric acid groups.<sup>14</sup>

Two nucleotides united through  
their phosphoric acid groupsTwo nucleotides united but not  
through their phosphoric  
acid groups

Until very recently it was conceded by everyone that if the nucleotide linkage of yeast nucleic acid is not through its phosphoric acid groups then it is naturally through the carbohydrate groups. That question is taken up in the following section.

## II. A COMPARISON OF THE RATE AT WHICH THE PURINES ARE SET FREE FROM YEAST NUCLEIC ACID WITH THE RATE AT WHICH THE PURINES ARE SET FREE FROM THE INDIVIDUAL PURINE NUCLEOTIDES

At the time the phosphoric acid studies were made with guanine nucleotide and adenine nucleotide, the rates at which they set free their guanine and adenine were ascertained. It is exceedingly rapid and the same for both purines and for yeast nucleic acid. The rapidity

<sup>13</sup> Levene: Journ. Biol. Chem., 1920, xli, 1.

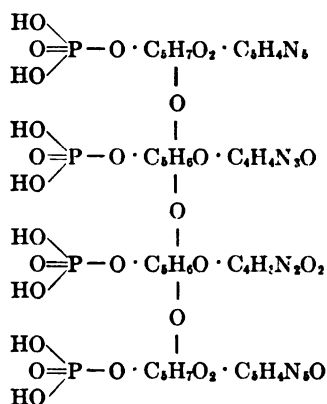
<sup>14</sup> The argument here used is essentially that which was employed for the same purpose by Jones and Read (Journ. Biol. Chem., 1917, xxix, 123). Their argument is today as sound as it was when they wrote.

is such that the time required to dissolve the nucleotide in the hydrolytic agent has to be considered and in the case of yeast nucleic acid the other products of hydrolysis make the determination of the liberated purines very difficult. But one can conclude from the data without hesitation that the purines are set free from nucleic acid and from the individual purine nucleotides with the same rapidity.

Again, the exceeding slowness with which the pyrimidines are split by hydrolysis from yeast nucleic acid makes quantitative work impossible. But the same slowness characterizes the pyrimidine nucleotides.

Therefore the argument which was used above to show that the nucleotide linkages of yeast nucleic acid do not involve the phosphoric acid groups, may now be used to show that the nucleotide linkages do not involve the purine groups nor probably the pyrimidine groups.

This leaves the carbohydrate groups and indicates the formula:



### III. A COMPARISON OF THE RATE AT WHICH PHOSPHORIC ACID IS SET FREE FROM ADENINE NUCLEOTIDE WITH THE RATE AT WHICH ADENINE IS SET FREE FROM ADENINE NUCLEOTIDE

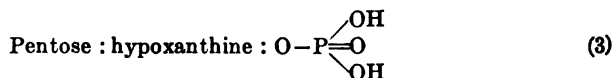
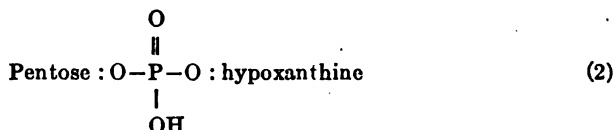
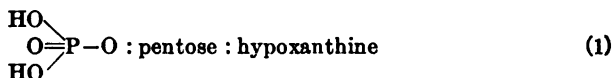
The longest known nucleotide (though not a nucleotide of yeast nucleic acid) is the substance that Liebig<sup>15</sup> discovered in meat extract and called inosinic acid. It was afterwards shown contemporaneously by Bauer<sup>16</sup> and by Neuberg and Brahn<sup>17</sup> that inosinic acid is com-

<sup>15</sup> Liebig's Annalen, 1847, lxii, 317.

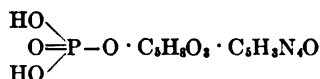
<sup>16</sup> Hofmeister's Beitr., 1907, x, 345.

<sup>17</sup> Biochem. Zeitschr., 1907, v, 439; Ber. d. d. chem. Gesellsch., 1908, xli, 3376.

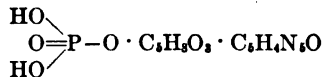
posed of the groups of three substances, viz., phosphoric acid, pentose and hypoxanthine. Hence inosinic acid may have any one of three structures, i.e., any one of the three groups may be the central group connecting the other two.



Liebig knew that inosinic acid is a dibasic acid. This excludes formula (2). Hauser<sup>18</sup> found that by acid hydrolysis inosinic acid loses its hypoxanthine much more rapidly than its pentose.<sup>19</sup> This excludes formula (3) and leaves the correct formula (1):



This method may be used to find the gross structure of any purine nucleotide (but obviously not of a pyrimidine nucleotide) and was applied to guanine nucleotide by Jones and Read<sup>20</sup> who found that the substance is a dibasic acid that forms a dibrucine salt and that, by acid hydrolysis, the nucleotide loses its guanine very much more rapidly than its phosphoric acid. These two facts necessitate the following arrangement of the three groups:



<sup>18</sup> Monatshefte f. Chem., 1895, xvi, 190.

<sup>19</sup> Hauser mistook the pentose for trioxypyruvic acid. Both substances have the formula  $\text{C}_5\text{H}_8\text{O}_7$ .

<sup>20</sup> Journ. Biol. Chem., 1917, xxxi, 337.

The curves of figure 3 are constructed from experimental data obtained with adenine nucleotide. The upper curve expresses the rate for adenine and the lower curve the rate for phosphoric acid. In five minutes three times as much adenine is set free as phosphoric acid: or, the liberation of adenine is nearly complete in thirty minutes, that of phosphoric acid only after two hours.

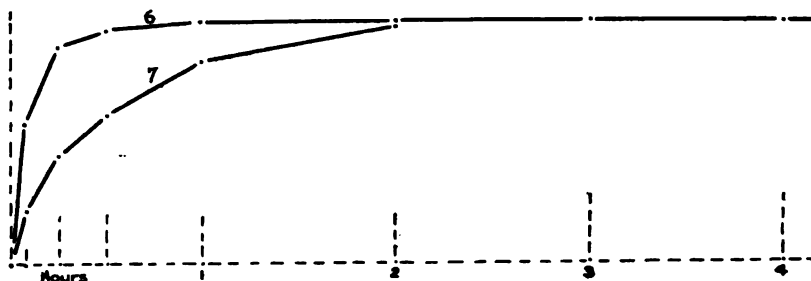
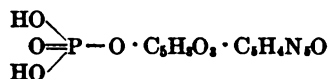


Fig. 3. The upper curve shows the rate at which adenine is set free from adenine nucleotide. The lower curve shows the slower rate for phosphoric acid.

Adenine nucleotide forms a dibrucine salt and conducts itself toward alkalis like a dibasic acid. Hence its groups must be arranged as indicated in the formula



#### IV. THE ACIDITY OF ADENINE NUCLEOTIDE

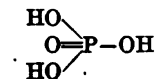
In the article that follows it will be assumed that the nucleotides conduct themselves like acids toward indicators and alkalis. One cannot work long with the nucleotides without knowing that this is true of them all. When a lead salt is decomposed with sulphuretted hydrogen the filtrate from the lead sulphide is strongly acid to litmus. As the matter is of considerable importance, however, an exact examination of adenine nucleotide was made.

A weighed portion of the nucleotide (50 mgm.) was covered with water and titrated with 0.104 N sodium hydroxide using phenolphthalein for the indicator; 2.69 cc. were required. A second portion of 50 mgm. was titrated using methyl orange for the indicator. Half as much alkali was required.

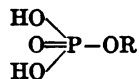
STANDARD ALKALI USED FOR 50 MGM. OF ADENINE NUCLEOTIDE		THEORETICALLY REQUIRED FOR	
Using phenolphthalein	Using methyl orange	Two equivalents of hydrogen	One equivalent of hydrogen
cc.	cc.	cc.	cc.
2.69	1.35	2.68	1.31

When free phosphoric acid is titrated with sodium hydroxide using methyl orange as an indicator exactly one equivalent of hydrogen is neutralized; but if phenolphthalein is used as an indicator, twice as much alkali is required, which of course corresponds exactly to two equivalents of hydrogen.

Adenine nucleotide therefore conducts itself toward alkalis exactly like free phosphoric acid. This is what one would expect a substance of its structure to do.



phosphoric acid



adenine nucleotide

### Experimental

Weighed portions of adenine nucleotide, placed in flasks provided with condensing tubes and treated with twenty parts of 5 per cent sulfuric acid, were heated in a boiling water bath. At various intervals from 15 minutes to 3 hours, a flask was removed from the water bath and both free phosphoric acid and free adenine were quantitatively determined as follows. The hot fluid was made alkaline with ammonia and treated with a slight excess of magnesia mixture. After standing 5 hours, the crystalline magnesium ammonium phosphate was filtered off, allowed to dry in the air and weighed. The weight was divided by the weight of the nucleotide used in the experiment to obtain the weight per gram of nucleotide, so that the results of various experiments could be directly compared with one another. It was also found convenient not to calculate the corresponding amount of phosphorus but to express the phosphoric acid throughout in terms of magnesium ammonium phosphate ( $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$ ).

The ammoniacal filtrate containing adenine was treated with a solution of silver nitrate in ammonia and the precipitated adenine-silver



compound was filtered off, thoroughly washed, suspended in water and decomposed with hydrochloric acid. After filtration from silver chloride, the acid fluid was evaporated to dryness (with the usual precautions) for the expulsion of all but a trace of free hydrochloric acid, and a solution of the residue in a little warm water was treated with a slight excess of picric acid. The precipitated crystalline adenine picrate was filtered off, allowed to dry in the air and weighed. This weight was divided by the weight of the nucleotide used in the experiment and the corresponding amount of adenine was calculated.

In addition, the total amount of phosphoric acid obtainable from the nucleotide after completely burning was determined and, for comparison, was expressed in terms of magnesium ammonium phosphate per gram of nucleotide.

The results are given in table 1.

Results obtained in a similar way by Jones and Read with guanine nucleotide are given in table 2, and for comparison the results obtained with yeast nucleic acid by Jones and Riley are given in table 3.<sup>21</sup>

TABLE 1  
*Adenine nucleotide*

NUCLEO- TIDE USED	TIME OF HYDROLYSIS	MAGNESIUM AMMONIUM PHOSPHATE $MgNH_4PO_4 \cdot 6H_2O$			ADENINE PICRATE		CALCULA- TED ADENINE	PER CENT OF THEO- RETICAL (0.3700)
		Obtained	Per gm. of nucleotide	Per cent of theo- retical (0.671)	Obtained	Per gm. of nucleotide		
0.2161	5 min.	0.0304	0.1409	21.0	0.1253	0.5800	0.2146	58.0
0.6204	15 min.	0.1915	0.3087	46.0	0.5569	0.8976	0.3330	90.0
0.6167	30 min.	0.2566	0.4160	62.0	0.5980	0.9696	0.3587	97.2
0.6302	1 hr.	0.3552	0.5637	84.0	0.6285	0.9973	0.3700	100.0
0.6111	2 hrs.	0.4084	0.6683	99.5	0.6017	0.9844	0.3652	98.7
0.6123	3 hrs.	0.4088	0.6678	99.7	0.6154	1.0051	0.3729	100.8
0.3033	4 hrs.	0.2023	0.6669	99.4	0.1114	0.9930	0.3674	99.3
0.3247	Total	0.2159	0.6646	99.5				
0.3103	Total	0.2060	0.6639	98.9				
	Theoretical		0.6710	100.0		1.000	0.370	100.0

<sup>21</sup> Two values are the results of later experiments.

TABLE 2  
*Guanine nucleotide*<sup>22</sup>

NUCLEOTIDE USED	TIME OF HYDROLYSIS	MAGNESIUM AMMONIUM PHOSPHATE $MgNH_4PO_4 \cdot 6H_2O$			GUANINE		
		Obtained	Per gm. of nucleotide	Per cent of total	Obtained	Per gm. of nucleotide	Per cent of total
0.4234	5 min.	0.0499	0.1178	18.8	0.0835	0.1972	50.0
0.5030	15 min.	0.1348	0.2680	42.8	0.1747	0.3473	88.1
0.5000	30 min.	0.2004	0.4008	64.0	0.1901	0.3802	96.3
0.3487	1 hr.	0.1881	0.5395	86.2	0.1396	0.4002	101.5
0.3360	2 hrs.	0.2017	0.6000	95.8	0.1310	0.3899	98.9
0.3448	3 hrs.	0.2158	0.6259	100.0	0.1360	0.3944	100.0
0.4112	4 hrs.	0.2539	0.6174	98.6	0.1617	0.3932	99.8

 TABLE 3  
*Yeast nucleic acid*<sup>23</sup>

NUCLEIC ACID USED	TIME	MAGNESIUM AMMONIUM PHOSPHATE $MgNH_4PO_4 \cdot 6H_2O$						
		Obtained	Per gram	From pyrimidine nucleotides	I Per cent of half the total (0.295)	From purine nucleotides	II Per cent of half the total	Sum of I and II
1.0031	15 min.	0.1374	0.137	0.003	0.85	0.134	40.4	41.25
1.0001	30 min.	0.2090	0.209	0.005	1.70	0.204	61.3	63.0
0.8642	1 hr.	0.2315	0.269	0.010	3.40	0.259	87.8	91.2
0.9837	2 hrs.	0.3125	0.318	0.020	6.80	0.298	101.0	107.8
0.9251	3 hrs.	0.3038	0.329	0.030	10.20	0.299	101.0	111.2
1.0305	4 hrs.	0.3488	0.338	0.040	13.60	0.298	101.0	114.6
0.8333	5 hrs.	0.2876	0.345	0.050	17.00	0.295	100.0	117.0
0.9927	6 hrs.	0.3565	0.359	0.060	20.40	0.299	101.0	121.4
0.8179	Total	0.5112	0.590	half the total = 0.295				

 TABLE 4  
*Pyrimidine nucleotides*<sup>24</sup>

NUCLEOTIDES USED	TIME OF HYDROLYSIS	MAGNESIUM AMMONIUM PHOSPHATE $MgNH_4PO_4 \cdot 6H_2O$		
		Obtained	Per gm. of nucleotides	Per cent of total
1.0362	3 hours	0.0647	0.0624	10.0
0.3587	Total	0.220	0.624	

<sup>22</sup> Table of Jones and Read. Journ. Biol. Chem., 1917, xxxi, 337. The four-hour period is added.

<sup>23</sup> Data taken from article of Jones and Riley, Journ. Biol. Chem., 1916, xxiv, i, except 15-minute and 30-minute periods.

<sup>24</sup> From data of Jones and Read, Journ. Biol. Chem., 1917, xxxi, 39.

## THE ACTION OF BOILED PANCREAS EXTRACT ON YEAST NUCLEIC ACID

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Received for publication March 12, 1920

The earliest investigations of nucleic acid had shown that by mild acid hydrolysis, the purines are set free with part of the phosphoric acid and part of the carbohydrate; but that violent hydrolytic processes are required to set free the pyrimidines with the remainder of the phosphoric acid and carbohydrate. A great amount of ingenuity was therefore not required to formulate a hypothetical structure for nucleic acid. The substance must be composed of four complexes, all of which contain a group of phosphoric acid and a group of carbohydrate but each complex contains a different one of the four nitrogenous groups.

Change the word "complex" to the word "nucleotide" and the above becomes essentially the modern nucleotide theory of the constitution of yeast nucleic acid.

But one important matter concerning the constitution of yeast nucleic acid was not touched upon by the early investigators. At what points are the nucleotides joined together to form yeast nucleic acid or, in other words, what is the mode of nucleotide linkage in yeast nucleic acid? Without any experimental evidence it was finally agreed that the nucleotide linkage is through the phosphoric acid groups and this assumption remained undisturbed until Jones, Germann and Read furnished the experimental evidence to show that this mode of nucleotide linkage is not correct. The principal object of the present paper is to describe an experiment which proves this in a very simple and striking way.

Pig's pancreas contains a variety of active agents (ferments) which decompose nucleic and further act on its decomposition products. When an aqueous extract of pancreas is boiled, all of these active agents are destroyed but one, viz., the one that decomposes yeast nucleic acid into its nucleotides.

An aqueous extract of pig's pancreas was boiled and filtered. Yeast nucleic acid was added to the clear extract and the mixture was allowed to digest for 20 hours at 40°, when the nucleic acid had disappeared<sup>1</sup> and a mixture of the four nucleotides could be isolated.

This ferment (if it is a ferment) acts rather rapidly at first but more slowly afterwards. It is exceedingly more active at 40° than at 20° and exhibits about the same activity whether the solution be amphoteric, faintly alkaline or acid to litmus. It is not present in the liver nor the spleen and does not decompose thymus nucleic acid.<sup>2</sup> In the decomposition of yeast nucleic acid by this ferment neither phosphoric acid nor purine bases are set free and deaminization does not occur. *But when nucleic acid is converted into nucleotides by this active agent there is not the slightest change in the acidity of the solution.*

Yeast nucleic acid is represented below by two formulas which differ from one another in only one respect. Formula I has its nucleotide linkages through the phosphoric acid groups; formula II has its nucleotide linkages through the carbohydrate groups.

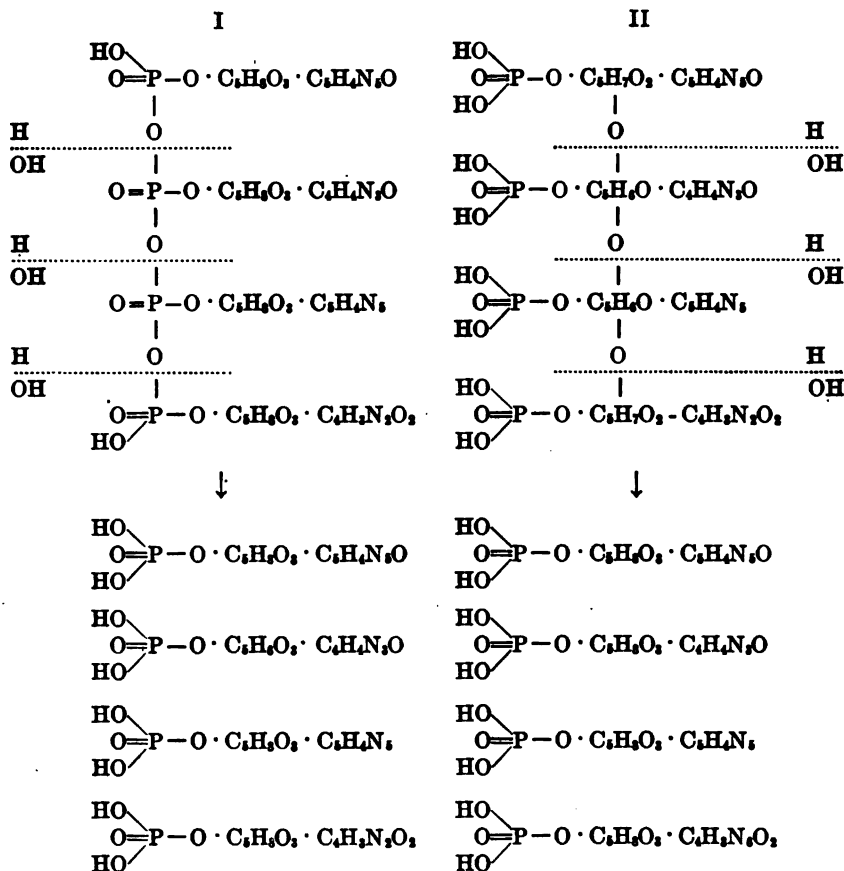
If formula I is correct, the conversion of nucleic acid into its nucleotides should be attended by a marked increase in acidity: but if formula II is correct, there should be no increase in acidity. If formula I correctly represents the structure of nucleic acid, then the increased acidity due to the decomposition of 2 grams of nucleic acid into its nucleotides should require about 8 cc. of 0.1 N sodium hydroxide for neutralization. But as a matter of fact it was not possible to demonstrate any change in acidity with sensitive indicators when 2 grams of nucleic acid was decomposed into its nucleotides.

This is a crucial experiment that decides against the phosphoric acid linkage and, I think, in favor of the carbohydrate linkage since the other possibilities are only of academic interest.

I cannot feel responsible for, nor even interested in the impossibility of two carbohydrate groups uniting with one another, and presume that such a union will be conceded without any proof if the other conceivable possibilities are excluded.

<sup>1</sup> The merest trace of nucleic acid can be detected in such a solution by the addition of sulphuric or hydrochloric acid.

<sup>2</sup> This is curious. An active agent is present in animal pancreas which is specifically adapted to plant nucleic acid. It suggests evolutionary matters.



## EXPERIMENTAL

A mixture of 2 kilos of carefully trimmed and ground pig's pancreas, 2 liters of water and 30 cc. of chloroform was allowed to digest for 12 hours at the room temperature in a tightly closed vessel with frequent and violent agitation. After the tissue had by this means become thoroughly penetrated with chloroform, the mixture was placed in a thermostat and allowed to digest at 40° for 2 days, when it was cooled and filtered. The clear, pale yellow filtrate was then boiled, filtered from a small coagulum and after cooling was preserved with chloroform for use in the following experiments. The data given are selected from a large amount of similar data.

a. 50 cc. of boiled extract + 0.250 gm. yeast nucleic acid<sup>\*</sup>

<sup>\*</sup> In all experiments the digesting material was preserved with chloroform.

- b. 50 cc. of boiled extract + 0.375 gm. yeast nucleic acid
- c. 50 cc. of boiled extract + 0.500 gm. yeast nucleic acid
- d. 50 cc. of boiled extract + 0.750 gm. yeast nucleic acid
- e. 50 cc. of boiled extract + 1.000 gm. yeast nucleic acid
- f. 50 cc. of boiled extract + 1.250 gm. yeast nucleic acid
- g. 50 cc. of boiled extract + 1.500 gm. yeast nucleic acid

All were digested at 40°. After 24 hours *a*, *b*, *c*, *d* and *e* gave no cloud with  $\text{H}_2\text{SO}_4$ ; *a* and *b* gave nothing with  $\text{HCl}$ , but *c*, *d* and *e* gave a slight cloud. After 48 hours *a*, *b* and *c* gave nothing with  $\text{HCl}$ ; *d* and *e* only an opalescence; *f* and *g* a faint cloud.

- a. 50 cc. of boiled extract + 0.750 gm. yeast nucleic acid at 20°
- b. 50 cc. of boiled extract + 0.750 gm. yeast nucleic acid at 40°

After 12 hours sulphuric acid gave a dense precipitate with *a* but nothing with *b*.

- a. 25 cc. of boiled extract + 0.375 gm. yeast nucleic acid
- b. 25 cc. of boiled extract + 0.375 gm. yeast nucleic acid
- c. 25 cc. of boiled extract + 0.375 gm. yeast nucleic acid
- d. 25 cc. of boiled extract + 0.375 gm. yeast nucleic acid

*a* was made amphoteric to litmus; *b* was made faintly alkaline; *c* was markedly alkaline; *d* was left acid. All behaved about alike. After 20 hours digestion at 40° all gave a faint cloud with  $\text{H}_2\text{SO}_4$  but nothing after 36 hours.

- a. 50 cc. of boiled pancreas extract + 0.750 gm. yeast nucleic acid
- b. 50 cc. of boiled pancreas extract + 0.250 gm. thymus nucleic acid
- c. 50 cc. of boiled spleen extract + 0.250 gm. yeast nucleic acid
- d. 50 cc. of boiled liver extract + 0.250 gm. yeast nucleic acid
- e. 50 cc. of phosphate mixture (pH = 6.4) + 0.250 gm. yeast nucleic acid.

Digested at 40°. After 19 hours *a* failed to give a cloud with  $\text{H}_2\text{SO}_4$  but *b*, *c*, *d* and *e* all gave dense precipitates with  $\text{H}_2\text{SO}_4$  even after 48 hours.

Three cubic centimeters of boiled pancreas extract were diluted with 10 cc. of water and treated with 4 drops of a solution of brom-cresol purple. On comparison of the color with a set of standard colors its acidity (pH) was found to be between 6.0 and 6.4 (about 6.2). A larger quantity of the extract was then titrated with 0.1 N sodium hydroxide to an acidity (pH) of 6.4. Changes in the acidity of such a solution can easily be detected with brom-cresol purple.

One and one-half gram of yeast nucleic acid were dissolved in 75 cc. of the above extract and the solution was titrated with 0.1 N sodium hydroxide to an acidity of 6.4, i.e., until the color which it gave with 4 drops of brom-cresol purple exactly matched the color similarly given by the extract. The extract and the solution were then digested at

40° for 48 hours. The nucleic acid had entirely disappeared and the color produced by brom-cresol purple with the solution exactly matched the color produced with the extract. The acidity of both solution and extract had not changed from 6.4. A trace of 0.1 N hydrochloric acid caused a very perceptible change in the acidity of the solution.

This experiment confirms many less accurate ones that were made. In the tests for the influence of acidity on the activity of the ferment no faintly alkaline solution ever became amphoteric to litmus and no amphoteric solution ever became acid. In fact, no change at all in the tint given to litmus was ever noticed as digestion proceeded.

50 cc. boiled pancreas extract

50 cc. boiled pancreas extract + 0.750 gm. yeast nucleic acid

The two solutions were digested at 40° for 48 hours, when the nucleic acid had entirely disappeared.

Each solution was made alkaline with ammonia and treated at the boiling point with an excess of magnesia mixture, when perfectly white crystalline ammonium magnesium phosphate was precipitated. This was filtered the next day, allowed to dry and weighed.

$\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$  from the experiment 0.5474

$\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$  from the blank 0.5469

An experiment was made with boiled extract which involved 125 gm. of yeast nucleic acid. After the nucleic acid had disappeared by digestion at 40° the product was heated to boiling and treated with neutral lead acetate as long as the reagent gave a precipitate in the hot fluid. This precipitate which consists principally of lead phosphate was filtered off with a pump and the pale yellow filtrate was treated in the warm with more lead acetate. At first no precipitate was produced but after the addition of a sufficient excess of the reagent a copious granular precipitate was thrown down. After cooling, the precipitated lead salts of the nucleotides were filtered off, washed, suspended in warm water and decomposed with sulphuretted hydrogen. The filtrate from lead sulphide was evaporated at 45° under diminished pressure and the nucleotides were thrown out and dried with absolute alcohol. This product is undoubtedly identical with the mixture of nucleotides formerly obtained by Jones and Richards<sup>4</sup> from yeast nucleic acid by treatment with an extract of pig's pancreas that had previously been digested for a long time at 40°. It contains all four nucleotides and forms a mixture of crystalline brucine salts. As the separation of the nucleotides from one another is foreign to the principal point of this paper, the matter will be taken up later in a separate article.

<sup>4</sup> Journ. Biol. Chem., 1915, xx, 25.





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No. 2

## A STUDY OF FORCED RESPIRATION: EXPERIMENTAL PRODUCTION OF TETANY

SAMUEL B. GRANT AND ALFRED GOLDMAN

*From the Physiological Department of Washington University*

Received for publication March 15, 1920

### INTRODUCTION

~~... was estimated to a rather large amount~~  
~~... may~~

### ERRATUM

Vol. LI, No. 3, pp. 533-534. "The Effect of Vitamine Deficiency on Various Species of Animals. I. The Production of Xerophthalmia in the Rabbit," by Victor E. Nelson and Alvin R. Lamb. The illustrations for figures 1 and 3 are transposed. The legends are correct.

When reading legend of figure 1 cf. cut printed with figure 3; *vice versa*, when reading legend of figure 3, cf. cut printed with figure 1.

tained precipitated phosphates. ~~in caption~~

it was surmised that the overventilation, by washing out carbon dioxide of the blood, tended to make the blood more alkaline, and that in order to maintain a constant hydrogen ion concentration in the body, alkali was excreted by way of the kidneys. The present investigation was undertaken with the idea of determining this question.

### METHODS

The experiments were carried out on human subjects according to the following plan. During the period of forced respiration the subject, with thoracic and abdominal pneumographs attached, was lying on his

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### INTRODUCTION

Tetany is a disease which has been subjected to a rather large amount of experimental study in recent years, the fact that its symptoms may be so readily produced by parathyroidectomy in animals, and the question of the relation of parathyroid tetany to idiopathic tetany in man, making it a particularly inviting field for experimental work. In the course of the work about to be described, it was found that all the essential symptoms of tetany could be produced in the human subject by forced respiration.

It was noted during the course of a series of experiments in which the subject was required to go through a period of increased respiration, that the urine collected at the end of this period was cloudy, and that the cloudiness was due to the fact that the urine was alkaline and contained precipitated phosphates. In explanation of these observations it was surmised that the overventilation, by washing out carbon dioxide of the blood, tended to make the blood more alkaline, and that in order to maintain a constant hydrogen ion concentration in the body, alkali was excreted by way of the kidneys. The present investigation was undertaken with the idea of determining this question.

### METHODS

The experiments were carried out on human subjects according to the following plan. During the period of forced respiration the subject, with thoracic and abdominal pneumographs attached, was lying on his

back, and breathed as deeply as possible in time with a metronome at the rate of about 14 per minute. This was continued until symptoms of tetany developed, usually for from 15 to 60 minutes. The pneumographs recorded the depth of respiration on smoked paper, within sight of the subject, who was thus better able to maintain the deep breathing. In the later experiments, when the subjects had become accustomed to forcing respiration, the pneumographs were discarded. The metronome, however, was used throughout to prevent the rate of breathing from becoming involuntarily too slow.

The alveolar carbon dioxide tension was measured at the beginning and at the end of each experiment, the Plesch (1) bag method of collecting the air and the Marriott (2) colorimetric method of determining carbon dioxide tension being used. In some of the experiments the alveolar carbon dioxide tension was determined at intervals throughout the period of forced respiration, and afterwards until it had returned to normal.

The urine secreted during a measured period of time just before the experiment was collected, and also that for the period during which the deep breathing occurred, and in some cases at fixed intervals subsequently until the urine had returned to normal. Each specimen was analyzed as to hydrogen ion concentration, titratable acidity and ammonia content. The hydrogen ion concentration was determined by the method of Henderson and Palmer (3). The acidity was titrated with 0.1 N NaOH, using phenolphthalein as the indicator. Ammonia was determined by the Folin permutit (4) method.

Twenty cubic centimeters of blood were drawn with a syringe from the veins at the elbow just before and at the end of forced respiration, and the hydrogen ion concentration, carbon dioxide capacity and calcium content were determined. The hydrogen ion concentration was determined by the dialyzing and colorimetric method of Levy, Rowntree and Marriott (5). Samples of blood immediately upon being collected and before coming in contact with the air, were put into the collodion sacs and dialyzed. Specimens of oxalated whole blood were also used in several cases. The carbon dioxide capacity of the blood was determined by the method of Van Slyke (6), and calcium was determined by the Lyman (7) method, both whole blood and serum being used.

## RESULTS

*Tetany in forced respiration.* Symptoms of tetany developed in each of twenty-four experiments, with the exception of the one control. The diagnosis was based on the presence of carpopedal spasm, Chvostek's sign, Trousseau's sign, Erb's sign of increased electrical irritability, and in one instance a tetanic convulsion.

Attention was drawn to this condition in the first experiment. The subject, S. G., breathed as deeply as possible for sixteen minutes, at the rate of 15 per minute. About ten minutes after the start his fingers began to tingle. At the end of fifteen minutes the muscles of his face felt stiff, and there was some difficulty in articulation. In the second experiment, A. G., subject, tingling in the fingers was noted. When at the end of forty minutes an attempt was made to determine the blood pressure, inflation of the arm band caused the hand immediately to go into a typical spasm of tetany. The fingers were flexed at the metacarpophalangeal joints; the second and third phalanges remained extended at the phalangeal joints; all the fingers, especially the thumb, were adducted and the hand was flexed at the wrist. The spasm could be voluntarily overcome by active motions, but as soon as these ceased the hand returned to the former position. Generally the spasm relaxed almost immediately after cessation of the forced breathing, but sometimes it persisted for as long as three minutes. In subsequent experiments the other signs and symptoms of tetany noted above were brought out.

Two subjects were used throughout the course of the experiments, and it was found that they differed somewhat in their reaction. Subject S. G. developed, after breathing deeply for about ten minutes, spasticity of the facial muscles. Chvostek's sign for tetany could then be easily elicited, i.e., tapping a branch of the facial nerve caused spasmodic contraction of the facial muscles. As the deep breathing continued this spasticity increased, until finally the muscles of the face, especially those about the mouth, contracted spontaneously, causing puckering of the lips and decided difficulty in articulation. In the meantime the hands had begun to tingle and feel numb, and finally, after the spasm of the face was well marked, they spontaneously went into the typical spasm described above. Chvostek's sign remained positive after forced respiration had ceased, and a slight reaction could be obtained at least twenty minutes after the end of the experiment. In subject A. G. the facial phenomenon was not nearly so marked, but the spasm of the hands was greater. He often noted a slight headache

soon after deep breathing started. In about fifteen minutes tingling of the hands began, and this gradually became more intense until the hands suddenly went into a very marked spasm. Trousseau's sign could usually be obtained several minutes before the onset of the spontaneous spasm. When the facial nerve was tapped only a slight twitching of the muscles occurred and this was usually not obtained until the carpal spasm was intense, and not always then. No spontaneous facial spasm occurred as in subject S. G. Spasm of the feet took place in two experiments in which A. G. was subject. The feet were extended, the first phalanges of the toes flexed and the second and third extended.

Tingling in the abdominal and thoracic muscles was noted by both subjects on several occasions, when the period of deep breathing was prolonged.

The electrical irritability of the muscles of the forearm, or Erb's sign, was tested in two experiments, and in both it was found that contraction occurred with a much smaller current at the end of deep respiration than before. The indifferent electrode was fastened to the upper part of the right arm, and the stimulating electrode was applied over the ulnar nerve just above the elbow, the same point of stimulation being used before and after forced breathing. The number of milliamperes of current necessary to cause contraction with the cathodal opening, cathodal closing, anodal opening and anodal closing stimuli before and at the end of deep respiration in experiment 18 were as follows:

*Experiment 18*

	o o c	c c c	a o c	a o o
Before deep respiration.....	5.5	2.5	2.5	2.0
	OC TETANY			
At end of deep respiration.....	1.25	0.5	1.25	1.25
In experiment 5 they were:				
Before deep respiration.....			3.5	1.8
At end of deep respiration.....			0.5	0.4

A complete tetanic convulsion occurred in experiment 18, in which A. G. was subject. No note of the exact rate of respiration was made but it was faster than usual. After breathing very deeply for thirty minutes he suddenly went into a complete tetanic convulsion. At the onset he involuntarily gave a loud, high-pitched scream, probably due



to contraction of the muscles of respiration and forcing of air out through contracted vocal cords. The entire body was rigid, all the muscles being contracted in tetanic spasm. The back was arched somewhat, and all extremities extended completely. Relaxation occurred within thirty seconds, and there was no further spasm. There were no ill effects following the convulsion.

The usual sequence of symptoms in the experiments is given in the following chart of a typical experiment, together with some of the experimental data.

*Experiment 11. A. G., subject*

TIME	
Before	Alveolar CO <sub>2</sub> tension, 40 mm. Urine pH = 4.9 Blood pH = 7.4 Plasma CO <sub>2</sub> capacity, 63.6 vol. per cent
0 minute	Starts deep breathing—12 per minute
10th minute	Slight headache
13th minute	Tingling in hands
16th minute	Trousseau's sign obtained. Slight Chvostek
17th minute	Both hands tingle markedly
20th minute	Spasm of both hands
21st minute	Alveolar CO <sub>2</sub> tension 20 mm.
22d minute	Blood collected pH=7.5 Plasma bicarbonate 45.8 vol. per cent
23d minute	Spasm of feet. No facial spasm. Stops deep respiration
25th minute	Urine collected. pH=7.9

*Changes in the blood, urine and alveolar air.* Decided changes took place in the alveolar air, blood and urine as a result of the forced respiration. Alveolar carbon dioxide tension fell, the blood became slightly more alkaline, the urine became decidedly alkaline, the plasma bicarbonate was reduced, the ammonia excretion was diminished, and there was a slight increase in the calcium content of the blood.

*Alveolar carbon dioxide.* In fifteen experiments in which the alveolar carbon dioxide tension was measured it fell from an average of 42 mm. before to 21 mm. of mercury at the end of the period of deep breathing (see table 1). The fall was rapid at first and then became slower. With the termination of the period of forced breathing the tension rose at first rapidly and then more and more slowly, to almost reach normal in twenty minutes (see figs. 1, 2, 3, 4).

In experiment 17 the alveolar air was collected both by the Haldane (8) method, which gives alveolar air as determined by the tension of the gases in arterial blood, and the Plesch method, which gives alveolar air at the tension the gases are contained in venous blood. It was found that the difference in carbon dioxide tension as deter-

TABLE 1

EXPERIMENT	ALVEOLAR CO <sub>2</sub> TENSION		LENGTH OF PERIOD OF DEEP RESPIRATION	SUBJECT
	Before deep respiration	At end of deep respiration		
			minutes	
1	40	22	16	S. G.
2	35	18	50	A. G.
3	38	18	32	A. G.
4	43	22	15	S. G.
5	40	22	25	A. G.
6	40	25	25	S. G.
8	45	22	25	S. G.
9	41	24	28	A. G.
10	45	22	20	S. G.
11	40	20	23	A. G.
12	45	25	20	S. G.
13	37	20	28	A. G.
14	45	21	37	A. G.
15	45	20	23	S. G.
16	45	22	23	S. G.
Average . . . .	41.8	21	25.9	

TABLE 2

TIME AFTER START OF DEEP RESPIRATION	ALVEOLAR CO <sub>2</sub> TENSION (MM.Hg.)		DIFFERENCE IN TENSION
	Plesch	Haldane	
minutes	mm.	mm.	mm.
0	45	37	8
5.5	31	23	8
11.5	26	19	7
16.0	23	18	5
21.5	22	18	4

mined by these two methods was less at the termination of the period of exaggerated respiration than it had been at the beginning, as shown in table 2.

*Hydrogen ion concentration of the blood.* This was determined in five experiments, and the average fall was from pH 7.41 before to pH 7.57

at the end of deep breathing (see table 3). These determinations were made on fresh blood directly from the vein, before clotting occurred. In three experiments the hydrogen ion concentration of oxalated blood was determined. Loss of carbon dioxide to the air could not be so readily prevented when this was done, but the blood was placed in a narrow test tube from the syringe, corked, gently mixed with oxalate crystals, and then dialyzed at once in the deep narrow collodion sacs. The results were as in table 4. The hydrogen ion concentration of blood may be calculated from the carbon dioxide capacity of the plasma

TABLE 3

EXPERIMENT	pH OF BLOOD		LENGTH OF PERIOD OF DEEP RESPI- RATION	SUBJECT
	Before deep respiration	At end of deep respiration		
			<i>minutes</i>	
9	7.40	7.60	26	A. G.
10	7.45	7.65	20	S. G.
11	7.40	7.50	23	A. G.
12	7.35	7.60	20	S. G.
13	7.45	7.50	28	A. G.
Average.....	7.41	7.57	23.5	

TABLE 4

EXPERIMENT	pH OF OXALATED BLOOD		LENGTH OF PERIOD OF DEEP RESPI- RATION	SUBJECT
	Before deep respiration	At end of deep respiration		
			<i>minutes</i>	
11	7.45	7.65	23	A. G.
12	7.35	7.60	20	S. G.
13	7.35	7.50	28	A. G.

and the alveolar carbon dioxide tension, using Hasselbach's (9) formula. This was done in three experiments in which the required data had been obtained, and in each of these the blood was shown to have become more alkaline during deep respiration. In experiment 2 pH rose from 7.44 to 7.61, in experiment 12 from 7.34 to 7.48, and in experiment 13 from 7.40 to 7.55.

*Plasma bicarbonate.* The carbon dioxide combining power of the plasma was determined in four experiments. It fell from an average of 59.5 v.p.c. before to 44.9 v.p.c. at the end of deep respiration. The greatest fall was 17.8, the smallest 13.0 (see table 5).

TABLE 5

EXPERIMENT	CO <sub>2</sub> CAPACITY OF PLASMA		LENGTH OF PERIOD OF DEEP RESPIRATION	SUBJECT
	Before	At end of deep respiration		
	v.p.c.	v.p.c.	minutes	
11	63.6	45.8	22	A. G.
12	56.0	43.0	17	S. G.
13	52.9	38.7	27	A. G.
21	65.6	52.2	21	S. G.
Average . . . . .	59.5	44.9	21.75	

*Hydrogen ion concentration of urine.* The urine specimen collected after forced respiration was alkaline to litmus in twelve of thirteen experiments. The determination of the hydrogen ion concentration showed, in the average of thirteen experiments, pH 5.2 in the specimens voided before the period of exaggerated respiration, and pH 7.4 in the specimens voided after this period (see table 6).

TABLE 6

EXPERIMENT	pH OF URINE		LENGTH OF PERIOD OF DEEP RESPIRATION	SUBJECT
	Before deep respiration	After deep respiration		
			minutes	
1	6.4	6.9	16	S. G.
2	4.7	7.1	50	A. G.
3	5.5	7.4	32	A. G.
4	4.8	7.3	15	S. G.
5	4.7	7.9	25	A. G.
6	5.0	7.1	25	S. G.
8	4.7	7.7	25	S. G.
9	5.5	7.2	26	A. G.
10	5.2	7.8	20	S. G.
11	4.9	7.9	23	A. G.
12	4.8	7.1	20	S. G.
13	5.8	7.7	28	A. G.
16	5.7	7.2	23	S. G.
Average . . . . .	5.2	7.4	25.2	

*Titrateable acidity of urine.* In twelve experiments the acidity of the urine as titrated with 0.1 N sodium hydroxide, using phenolphthalein as the indicator, fell from an average of 7.54 cc. 0.1 N acid to 2.23 cc. per half-hour (see table 7).

TABLE 7

EXPERIMENT	TITRATED ACID		LENGTH OF PERIOD OF DEEP RESPIRATION	SUBJECT
	Of before urine; cc. 0.1 N per $\frac{1}{2}$ hour	Of after urine; cc. 0.1 N per $\frac{1}{2}$ hour		
	cc. 0.1 N	cc. 0.1 N	minutes	
2	10.93	2.80	50	A. G.
3	8.70	1.75	32	A. G.
4	9.37	1.68	15	S. G.
5	10.54	2.36	25	A. G.
6	10.05	1.46	25	S. G.
8	4.59	0.75	25	S. G.
9	6.34	2.14	26	A. G.
10	3.53	1.24	20	S. G.
11	5.40	1.67	23	A. G.
12	5.80	2.07	20	S. G.
13	7.88	1.74	28	A. G.
16	9.72	3.05	23	S. G.
Average . . . . .	7.54	2.23	26	

*Ammonia excretion.* The ammonia content of the urine was determined in twelve experiments. The average decrease was from 13.18 mgm. ammonia nitrogen per half-hour before, to 5.57 mgm. ammonia nitrogen after forced respiration (see table 8).

TABLE 8

EXPERIMENT	AMMONIA		LENGTH OF PERIOD OF DEEP RESPIRATION	SUBJECT
	In before urine; mgm. NH <sub>3</sub> nitrogen per half-hour	In after urine; mgm. NH <sub>3</sub> nitrogen per half-hour		
	mgm.	mgm.	minutes	
2	16.45	7.85	50	A. G.
3	13.40	4.74	32	A. G.
4	14.20	5.32	15	S. G.
5	16.50	5.85	25	A. G.
6	9.00	3.90	25	S. G.
8	11.74	4.28	25	S. G.
9	17.40	10.13	26	A. G.
10	8.00	3.93	20	S. G.
11	12.00	7.20	23	A. G.
12	10.20	4.50	20	S. G.
13	14.25	4.13	28	A. G.
16	15.00	5.00	23	S. G.
Average . . . . .	13.18	5.57	26	

*Calcium content of blood.* Calcium was determined in whole blood in one experiment and in serum in three experiments, and in each case a small but definite increase in calcium content was found in the blood collected at the end of the period of deep respiration over that found in the blood before deep respiration. All determinations were run in duplicate, some in triplicate, and a blank was run in each case. The figures are given in table 9.

TABLE 9

EXPERIMENT	Ca CONTENT OF BLOOD		LENGTH OF PERIOD OF DEEP RESPIRATION	SUBJECT
	Before deep respiration	At end of deep respiration		
	mgm. per 100 cc.	mgm. per 100 cc.	minutes	
18 (Whole blood)	7.00	7.29	67	A. G.
19 (Serum)	11.81	13.19	51	S. G.
20 (Serum)	12.75	13.31	62	A. G.
21 (Serum)	12.84	13.44	21	S. G.

The blood taken from the veins at the end of deep respiration had a much brighter red color than normal venous blood.

*Control experiments.* It was necessary to show, *a*, that the intake of food was not influencing the results; and *b*, that the results could not be attributed to any factor other than the overventilation. In experiments 9, 10, 11 and 12 the subjects omitted breakfast on the morning of the experiment. There was no appreciable difference between the results in these experiments and any of the others except that the titratable acidity of the control urine specimens was less than in the other experiments.

In experiment 7 means were taken to prevent the subject from lowering the carbon dioxide content of the alveolar air. He breathed through a tube connected to a fifteen-liter bottle, while the observer at the same time breathed through a second tube connected to the bottom of the bottle. The resulting high carbon dioxide content of the air in the bottle prevented the subject from washing out the carbon dioxide from his blood, in spite of the forced respiration. Under these conditions the alveolar carbon dioxide tension was slightly higher at the end of the experiment than it had been before. The acidity and ammonia content of the urine increased slightly, rather than decreasing as in the other experiments, and the hydrogen ion concentration of the urine remained about the same. The figures are given in table 10.

No symptoms of tetany occurred in this experiment. The subject became very slightly cyanosed, and after the experiment was dyspnoeic for a few minutes. Breakfast was omitted on the morning of the experiment.

TABLE 10

*A. G. subject. Deep breathing 17 minutes*

URINE pH		URINE ACID (cc. 0.1 N)		NH <sub>3</sub> NITROGEN (mgm.)		ALVEOLAR CO <sub>2</sub>	
Before	After	Before	After	Before	After	Before	After
4.6	4.8	7.79	8.27	11.2	13.3	38	40

Four of the experiments may now be taken up in detail in order to illustrate more concisely the exact method of procedure and the uniformity of the results.

*Experiment 12. S. G., subject*

TIME	
	Alveolar CO <sub>2</sub> tension=45 mm.
	Urine pH=4.8
0 minutes	Blood pH=7.35
	Plasma CO <sub>2</sub> capacity=56 v.p.c.
10 minutes	Starts forced respiration
20 minutes	Chvostek's sign obtained. Alveolar CO <sub>2</sub> , 28 mm. Tingling in fingers
23 minutes	Spasm of facial muscles—Chvostek's sign marked.
	Trousseau's sign positive
27 minutes	Blood collected
	pH=7.60
	Plasma CO <sub>2</sub> capacity=43 v.p.c.
30 minutes	Alveolar CO <sub>2</sub> tension=25 mm. Stops forced respiration
40 minutes	Urine collected pH=7.1
45 minutes	Alveolar CO <sub>2</sub> tension=33 mm.
55 minutes	Alveolar CO <sub>2</sub> tension=37 mm.
1 hour 10 minutes	Urine collected pH=5.0
1 hour 15 minutes	Alveolar CO <sub>2</sub> tension=39 mm.
2 hours 10 minutes	Urine collected pH=5.0
2 hours 20 minutes	Alveolar CO <sub>2</sub> tension=40 mm.

The graph of this experiment (fig. 1) shows the synchronous changes in alveolar air, blood and urine. Time in minutes is plotted on the horizontal axis, and alveolar carbon dioxide tension in millimeters of mercury, plasma carbon dioxide capacity in volume per cent, hydrogen

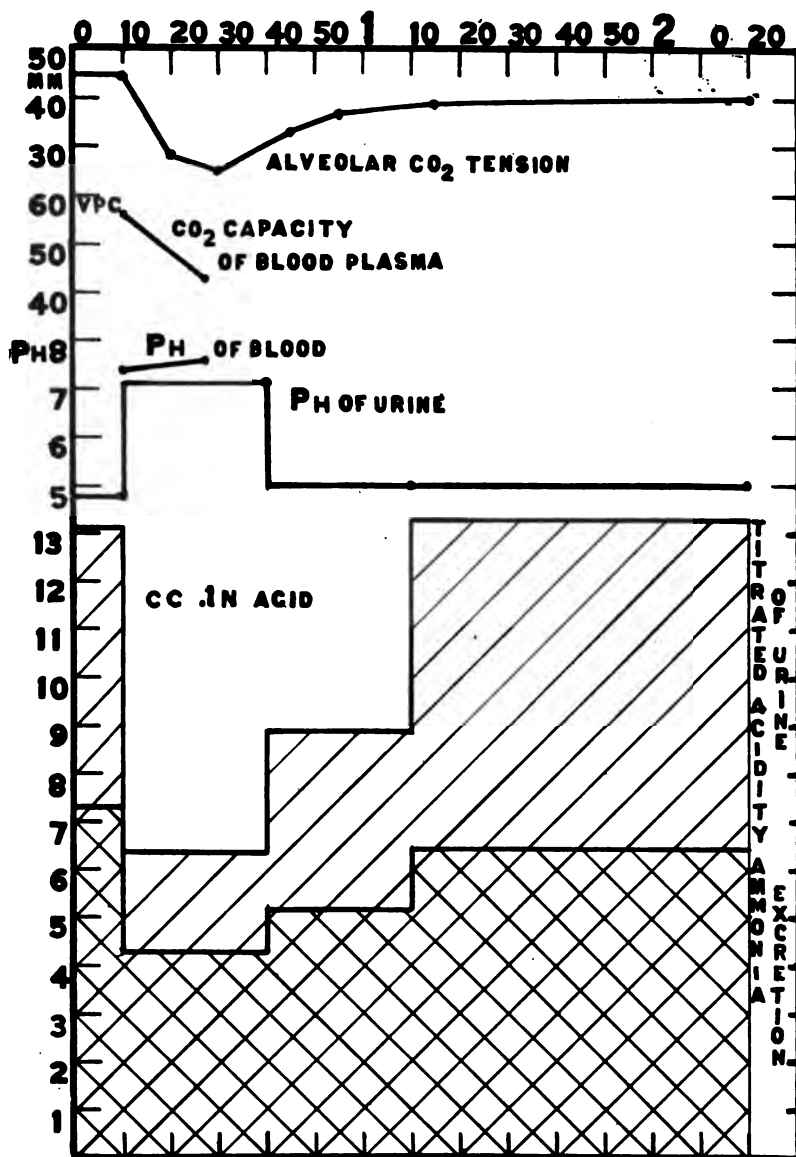


Fig. 1. Experiment 12. Forced respiration begins at 10 minutes and ends at 30 minutes.



ion concentration of blood and urine in terms of pH, and ammonia excretion and titrated acidity of urine per half-hour in cc. .IN solutions on the perpendicular axis. Deep breathing starts at 10 and ends at 30 minutes. As the alveolar CO<sub>2</sub> tension falls, the CO<sub>2</sub> capacity of the plasma falls, hydrogen ion concentration of blood and urine fall, excretion of ammonia decreases, and titrated acidity of urine falls. After deep breathing ceases, alveolar CO<sub>2</sub> tension rises, and corresponding changes occur in the urine.

*Experiment 13. A. G., subject*

TIME	
0 minute	Alveolar CO <sub>2</sub> tension=37 mm. Urine pH=5.8 Blood pH=7.45 Plasma CO <sub>2</sub> capacity=52.9 v.p.c.
10 minutes	Starts forced respiration
25 minutes	Slight headache
30 minutes	Alveolar CO <sub>2</sub> tension=23 mm.
31 minutes	Tingling in right foot. No Chvostek
32 minutes	Hands tingle
33 minutes	Tingling increased
34 minutes	Trousseau's sign markedly positive
35 minutes	Both hands in spontaneous spasm
36 minutes	Slight spasm of feet
37 minutes	Blood drawn pH=7.50 Plasma CO <sub>2</sub> capacity=38.7 v.p.c. Spasms of hand more intense
38 minutes	Stops forced respiration
40 minutes	Alveolar CO <sub>2</sub> tension=20 mm.
42 minutes	Urine collected pH=7.7 Temporary dizziness and trembling of hands on standing up after forced respiration
45 minutes	Alveolar CO <sub>2</sub> tension=28 mm.
50 minutes	Alveolar CO <sub>2</sub> tension=32 mm.
1 hour	Alveolar CO <sub>2</sub> tension=37 mm.
1 hour 23 minutes	Urine collected pH=6.9
1 hour 50 minutes	Urine collected pH=5.0

In the graph of this experiment (fig. 2) the same changes are shown as in figure 1, with the exception that the titrated acidity of the urine is not charted. Deep breathing starts at 10 and ends at 38 minutes. The third specimen of urine after forced breathing is more acid than the control urine specimen.

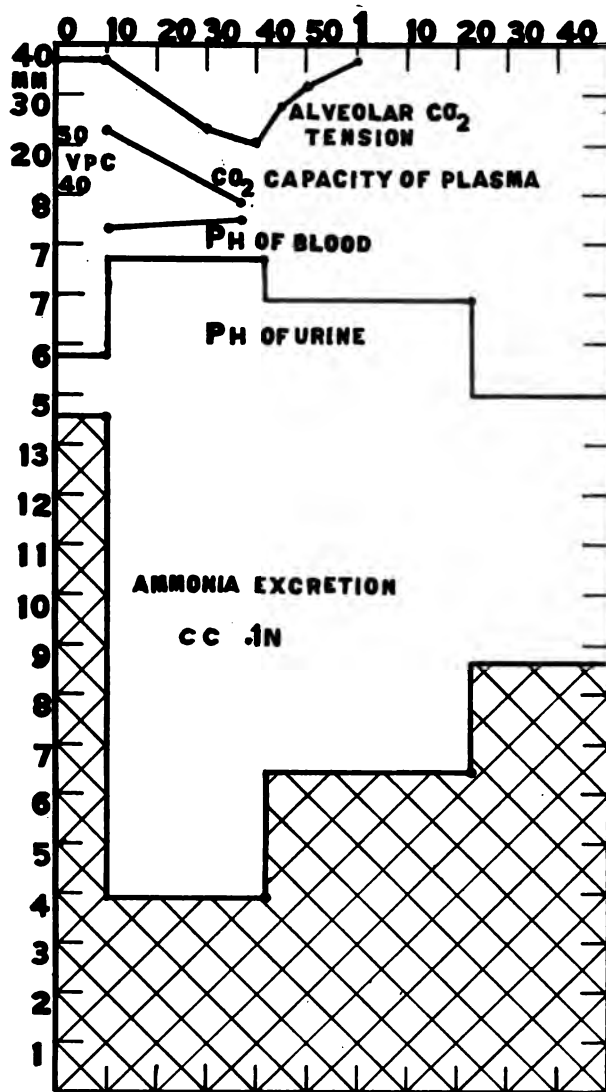


Fig. 2. Experiment 13. Forced respiration begins at 10 minutes and ends at 38 minutes.

*Experiment 16. S. G., subject*

TIME	
30 minutes	Urine collected pH=5.0
1 hour	Urine collected pH=5.7
1 hour 5 minutes	Alveolar CO <sub>2</sub> tension=45 mm.
1 hour 7 minutes	Starts forced respiration
1 hour 12 minutes	Alveolar CO <sub>2</sub> tension=31 mm.
1 hour 18 minutes	Alveolar CO <sub>2</sub> tension=26 mm.
1 hour 22 minutes	Tingling in hands
1 hour 23 minutes	Marked tingling in hands. Alveolar CO <sub>2</sub> tension=23 mm.
1 hour 26 minutes	Carpal spasm
1 hour 28 minutes	Alveolar CO <sub>2</sub> tension=22 mm.
1 hour 30 minutes	Stops forced respiration
	Urine collected pH=7.2
2 hours	Urine collected pH=6.8
2 hours 45 minutes	Urine collected pH=5.8
3 hours 45 minutes	Urine collected pH=4.8
4 hours 15 minutes	Urine collected pH=5.5

*Experiment 4. S. G., subject*

TIME	
20 minutes	Alveolar CO <sub>2</sub> =43 mm.
25 minutes	Urine collected pH=4.8
30 minutes	Starts forced respiration
38 minutes	Tingling and numbness in hands
40 minutes	Headache
41 minutes	Rigidity of facial muscles. Chvostek markedly positive, right and left
42 minutes	Carpal spasm both hands
44 minutes	Abdominal and thoracic muscles begin to tingle. No spasticity felt by observer
45 minutes	Stops forced respiration
	Alveolar CO <sub>2</sub> tension=22 mm.
55 minutes	Urine collected pH=7.3
	Chvostek weakly positive
	Alveolar CO <sub>2</sub> tension=32 mm.
1 hour 7 minutes	Chvostek negative
1 hour 15 minutes	Alveolar CO <sub>2</sub> tension=40 mm.
1 hour 25 minutes	Urine collected pH=4.8
1 hour 35 minutes	Alveolar CO <sub>2</sub> tension=43 mm.
2 hour 25 minutes	Urine collected pH=4.7
3 hour 25 minutes	Urine collected pH=5.2

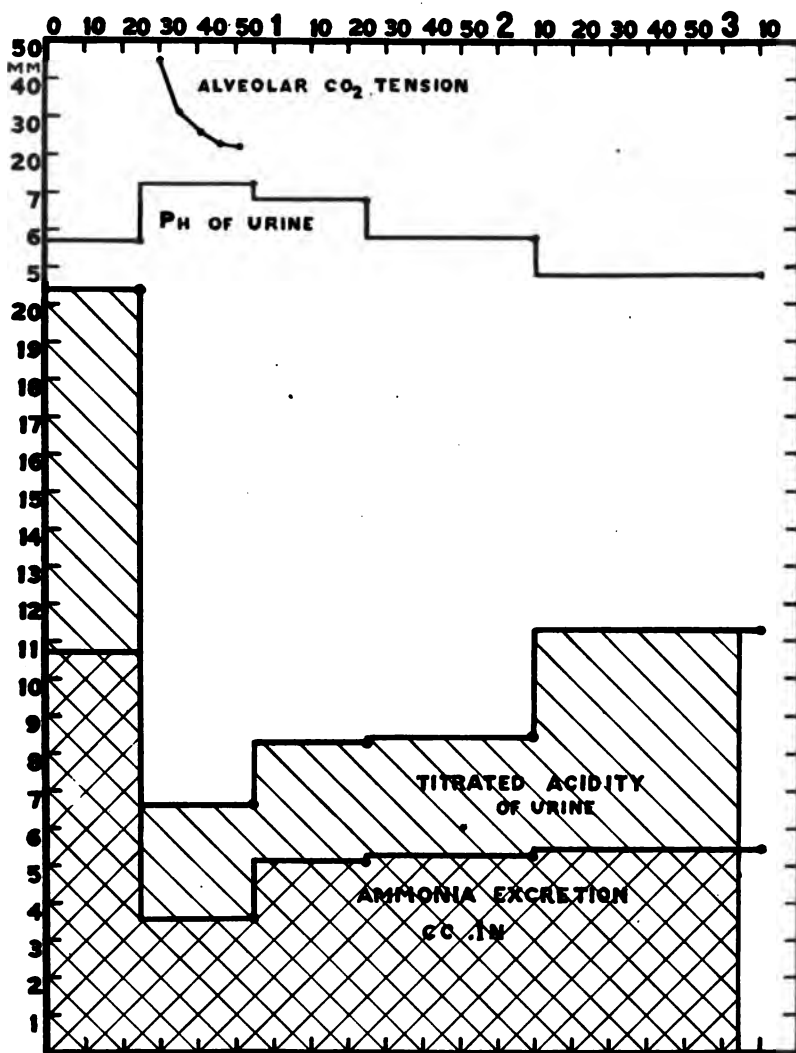


Fig. 3. Experiment 16. Forced respiration begins at 30 minutes and ends at 53 minutes.

Figure 3 is a graph of experiment 16. Deep breathing starts at 30 minutes and ends at 53 minutes. The fall in alveolar  $\text{CO}_2$  tension is very marked at first and then gradually becomes smaller as the low

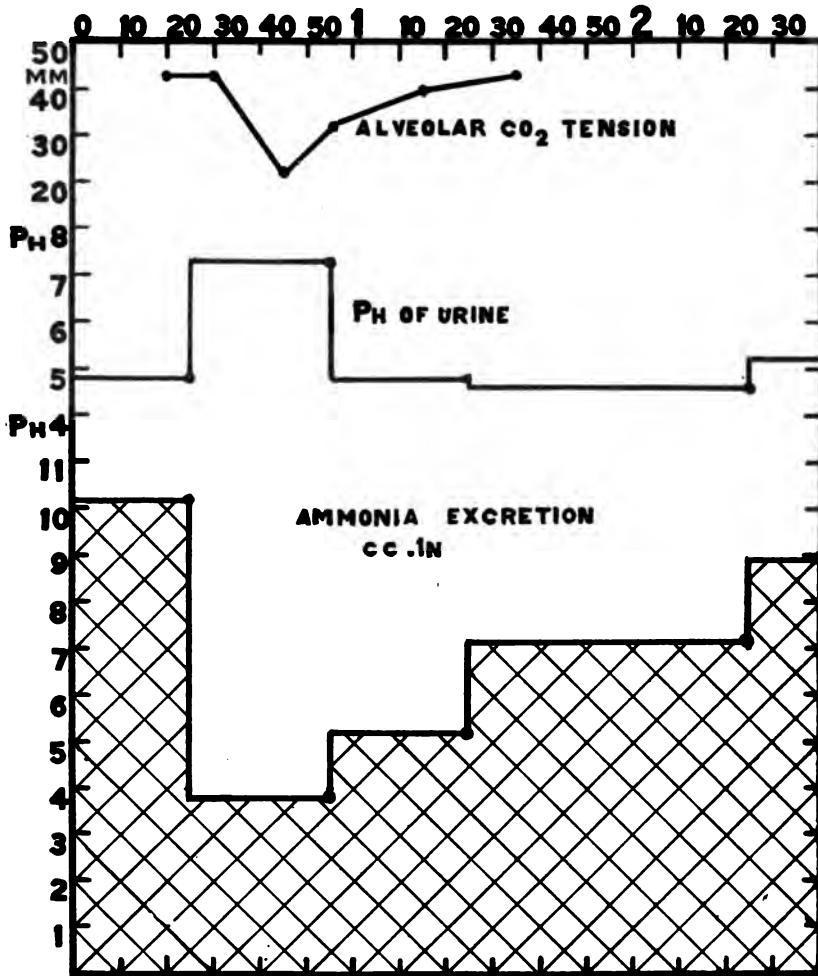


Fig. 4. Experiment 4. Forced respiration begins at 30 minutes and ends at 45 minutes.

level is reached. The urine pH reaches its control level 1 hour and 15 minutes after the forced respiration, while the specimen collected 2 hours 15 minutes after is more acid than the control one. The other changes are similar to those shown in figures 1 and 2.

The changes in experiment 16 (fig. 4) are similar to those described in the previous ones. The deep breathing begins at 30 and ends at 45 minutes. The return of the alveolar  $\text{CO}_2$  tension to the control level is shown, 60 minutes elapsing before this occurs. The ammonia does not reach the control level in 2 hours and 30 minutes.

#### DISCUSSION

Forced respiration, by washing out carbon dioxide from the alveoli, reduces the carbon dioxide content of the blood and so tends to make the blood more alkaline than normal. This reduction of carbon dioxide in the blood disturbs the  $\frac{\text{H}_2\text{CO}_3}{\text{NaH CO}_3}$  ratio upon which the hydrogen ion concentration depends, and on the reestablishment of this ratio,  $\text{NaH CO}_3$  passes out of the blood plasma into the tissues, and a considerable portion is excreted by way of the kidneys. Apparently,  $\text{CO}_2$  may be washed out of the blood more rapidly by overventilation, than  $\text{NaH CO}_3$  is decreased by excretion or other means, for on this assumption alone is it possible to account for the increased alkalinity actually found in blood during deep respiration. The diminution in ammonia formation is a further evidence of the effort of the body to compensate for the increased alkalinity of the blood. The lowered urine acidity is due partly to the increased alkali excretion, but probably largely to a retention of acid radicals.

In view of these considerations it seems logical to conclude that there is a true condition of "alkalosis" in the body during overventilation; the body fluids are actually more alkaline than normal. It is realized that the term "alkalosis" is a poor one, but we are using it strictly in the sense of an actual change in hydrogen ion concentration. In this condition of alkalosis we have an actual reduction of the "alkaline reserve," due to the elimination of  $\text{NaHCO}_3$  from the body fluids in an effort to maintain a constant hydrogen ion concentration. The coexistence of a more alkaline blood and a reduced alkaline reserve is another example of the inadequateness of the terms "alkalosis" and "acidosis."

The question now arises, is it possible to establish an etiological relationship between this condition of alkalosis and the tetany which develops during forced respiration? This may be discussed in connection with the views as to the cause of tetany that are held at the present time, the two principal ones of which are, first, that it is due to a calcium deficiency in the blood; and second, that it is due to a disturbance of the acid-base equilibrium in the body.

The literature contains some references to this problem. Thus, Wilson, Stearns and Thurlow (10) and Wilson, Stearns and Janney (11) have found that in parathyroidectomized dogs, just before an attack of tetany, a period of "alkalosis" develops, which is neutralized by the acid substances that are produced during tetany. They base their conclusion that an "alkalosis" obtains on the study of the dissociation constant of the oxyhemoglobin, the  $\text{CO}_2$  content of alveolar air, the hydrogen ion concentration of blood, the ammonia content of urine and the total acidity and hydrogen ion concentration of urine. Injections of acid and also of calcium salts relieve the tetany. They state that possibly part of the beneficial action of the calcium salts may be due to a relative increase in acid radicals caused by their administration. This might be brought about by the formation of  $\text{Ca}_3(\text{PO}_4)_2$  from the carbonates, thus liberating  $\text{HCl}$ .

McCann (12) finds after parathyroidectomy (and following operations on the stomach which exclude the acid secreted from the duodenum) that there is a marked increase in the  $\text{CO}_2$  combining power of the plasma, coincident with the development of tetany and concludes that tetany is a condition of alkalosis in which a disproportion between the rates of secretion of acids and alkalis by the gastro-intestinal tract may be a factor. He points out that clinically gastric tetany is most apt to occur in those cases in which there is some pyloric obstruction, so that the acid gastric secretion does not pass into the duodenum to excite the alkaline intestinal secretion, and that this might be expected to leave the blood with an excess of basic substances. MacCallum (13) and his coworkers in a paper just published state that when the pylorus of a dog is occluded, and only distilled water is introduced into the duodenum, the gastric mucosa continues to secrete hydrochloric acid. At the same time the alkali reserve increases and tetany-like symptoms appear. This can be prevented by the introduction into the duodenum of sodium chloride or the symptoms may be made to disappear by the intravenous injection of sodium chloride. Injection of hydrochloric acid is not so successful. Calcium determinations by a dialyzing method showed no change in the calcium content of plasma. The authors think that possibly the disturbed equilibrium of acids and bases in itself is the cause of the symptoms.

Cases of tetany following therapeutic administration of sodium bicarbonate have been reported. Howland and Marriott (14) cite three cases in children, all of which were associated with a marked diminution of the calcium content of the serum. Harrop (15) reports a case in an

adult who developed tetany after the intravenous injection of sixty grams of sodium bicarbonate. There was no associated diminution in calcium.

Binger (16) has shown that when sodium phosphate in sufficient quantity and at the right reaction is injected into dogs intravenously, symptoms of tetany result, associated with a marked decrease in the calcium content of the serum. But if the solution injected has a hydrogen ion concentration greater than  $10^{-8}$ , no tetany occurs even though there is a diminution in calcium.

Ammonia poisoning produces symptoms strikingly similar to those of parathyroid tetany. Marfori (17) found after intravenous injections of the carbonate, lactate and tartrate of ammonia in dogs, tremors, muscle twitchings, even tetany and opisthotonus, and irregular respiration, vomiting, etc. MacCallum and Voegtlin (18) found increased ammonia content of the blood in parathyroid tetany as did also Carlson and Jacobson (19) in their earlier work. Later, however, using improved methods, Carlson and Jacobson could find no change in ammonia content of blood in parathyroidectomized dogs.

The fact that increased respiration is associated with changes in the reaction of the urine had been previously noted by Hasselbach (20). He found that when the alveolar  $\text{CO}_2$  tension was raised a few millimeters by the influence of morphine on the respiratory center, the urine became very slightly more acid, and that when the alveolar  $\text{CO}_2$  tension was decreased by stimulation of respiration by light baths, the urine became barely perceptibly more alkaline.

After the present work had been started, Leathes (21) published a paper in which it was stated that increasing the respiration voluntarily for from one-half to one hour increased the alkalinity per cent of the urine from about 35 per cent to about 90 per cent. There was no mention made of symptoms of tetany or other changes during deep respiration.

Yandell Henderson and Haggard (22) found a great reduction of  $\text{CO}_2$  capacity of blood plasma of dogs in ether hyperpnoea and in excessive pulmonary ventilation by artificial respiration, together with a concomitant fall in blood pressure. They state that when the  $\text{CO}_2$  capacity of plasma falls below 33 to 36 v.p.c., the process becomes irreversible, and the animal dies in shock. The lowest point to which  $\text{CO}_2$  capacity of plasma fell in our work, in those experiments in which it was determined, was 43 v.p.c. Symptoms of tetany are not mentioned in their paper.



Stein (23) in discussing tetany as a sequel of gynecological operations cites fifteen cases in which it occurred. In four of these the symptoms of tetany occurred during the operation, and disappeared quickly without recurrence later. In three cases the tetany occurred during light ether anesthesia, one of these as narcosis was being induced, while the patient was still conscious. In the fourth case tetany occurred after the removal of eight liters of fluid from the peritoneal cavity by paracentesis, and lasted for fifteen minutes. It seems not unlikely that tetany in these instances may have been due to overventilation.

McClendon (24) has studied the effect of hydrogen and hydroxyl ions on the pulsation rate of the jelly fish and the heart of the conch, and finds that the activity may be inhibited by increasing H-ions in the surrounding fluid or perfusing liquid, as the case may be, and augmented by increasing the OH-ions. Biedermann (25) found that the heightened excitability of voluntary muscle produced by NaCl solution is greatly increased by the addition of  $\text{Na}_2\text{CO}_3$ , the muscle then contracting rhythmically and exhibiting a striking increase of response to artificial stimuli. Strong solutions of  $\text{Na}_2\text{SO}_4$  and dilute NaOH act in a similar manner but to a less degree. He seems to attribute this to a specific effect of the sodium ion, but it may be that part of the action is due to the alkalinity of the solutions in the case of  $\text{Na}_2\text{CO}_3$  and NaOH.

Recently a number of investigators have come to regard tetany as being due to a decrease in the calcium content of the blood. Thus MacCallum and Voegtlin (18) and MacCallum and Vogel (26) have shown that in the tetany of parathyroidectomized dogs there is a marked diminution of blood calcium. Howland and Marriott (27) have found that in infantile tetany the calcium content of the serum is greatly reduced, the average being about 5.6 mgm. per 100 cc. compared to 10 or 11 mgm. in the normal child. The administration of calcium promptly relieved the symptoms of tetany. On the other hand we have found in tetany of forced respiration no diminution of calcium content of the blood or serum. There is, as a matter of fact, a slight increase in calcium in every instance in blood specimens taken at the onset of tetany over those obtained under normal conditions of respiration. This may not necessarily mean that calcium plays no definite part in the production of the tetany of increased respiration. Thus Barille (Wells, 28) states that a decrease of  $\text{CO}_2$  in the blood results in precipitation of calcium. And Marriott (29) has shown that when artificial blood is brought into equilibrium with  $\text{CO}_2$  at 30 mm. Hg. tension, calcium is precipitated in a perceptible cloud. The possibility must

therefore be considered that when the  $\text{CO}_2$  of blood is reduced by over-ventilation a portion of the calcium is "precipitated" or in some way rendered inactive, though still present in the circulating blood. On the basis of this supposition the increase of calcium which we have found might be interpreted as being due to a compensatory mechanism on the part of the body to make up for a loss of active calcium. The calcium in our subjects was not precipitated in the blood, however, for centrifuging the serum specimens did not affect the analysis. It is conceivable, however, that the calcium is "precipitated" as very minute particles which are held in solution by the colloids of the serum, in which case centrifuging might have no effect on the calcium present. A further attempt to determine whether calcium plays a part in the production of tetany of overventilation will be made in a future investigation.

It may not be inappropriate to refer in this connection to the hypothesis advanced by Stoeltzner (30) attributing tetany to an accumulation of calcium in the blood. He found support for his views in some observations on the electrical reactions of peripheral nerves in infants after the administration of calcium. But these observations of Stoeltzner's have failed of confirmation.

Tetany occurs under so many circumstances that it probably cannot be attributed to a single etiological factor. It is more likely that it must be regarded as a symptom complex, and possibly any condition that heightens the irritability of peripheral nerves may cause it. Observation seems to indicate that alkalosis may be such a cause, and it probably is the underlying factor in the tetany that develops as a result of forced respiration.

#### SUMMARY

1. Forced respiration causes symptoms of tetany to occur in the human subject; these include carpopedal spasm, Chvostek's sign, Trousseau's sign, Erb's sign, and in one instance a tetanic convulsion.
2. As a result of the fall of alveolar  $\text{CO}_2$  tension produced by over-ventilation, there is a reduction in the hydrogen ion concentration of the blood, a reduction of the  $\text{CO}_2$  capacity of plasma, a change in the reaction of the urine to the alkaline side, a decreased excretion of ammonia, and a slight increase in the calcium content of the serum.
3. The underlying factor in the tetany of forced respiration is the alkalosis.

We wish to express our deep appreciation of the invaluable help and interest in this work given us by Dr. Joseph Erlanger.

We are also indebted to Dr. P. A. Shaffer and Dr. W. McK. Marriott for their kindly interest throughout the work.

*Note.* After the completion of this paper, several important references bearing upon our results were found in a journal which had been temporarily lost from the library. H. M. Vernon (31) in some observations on the production of prolonged apnoea in man says, "I found that after about 6 minutes of forced breathing the muscles of my hands passed into a condition of tonic rigidity, and for the first one and one-half minutes of the subsequent apnoea they were completely paralyzed. My other muscles were apparently unaffected." In no case in our experiments were the hands paralyzed, for we have always been able to voluntarily overcome the spasm, and this is also true of the spasm of idiopathic tetany.

Yandell Henderson (32) had a large number of men undergo voluntary hyperpnoea for short periods of time (usually 45 to 90 seconds) and makes the following statement. "Several of the subjects after vigorous hyperpnoea for 1 or 2 minutes, experienced in varying degrees the phenomenon mentioned by Vernon. . . . Even more common in our experience is a prickling sensation in the legs and arms, or in some cases in the entire body and face, somewhat similar to a hand or foot 'asleep.'" In one case, after vigorous hyperpnoea for two minutes a "shivering fit" came on, similar to that seen in a chill, and involving apparently all the muscles.

Hill and Flack (33), in determining the effect of oxygen inhalation on muscular work, had men undergo voluntary hyperpnoea for short periods. They observed feelings of numbness in the limbs, with a spastic state of the hands. One subject felt "twitching of the facial muscles." In another the "mouth was felt pursed up in the form of an O, so that speaking became difficult," and the muscles surrounding the eyes seemed contracted, so that the eyes were difficult to open fully.

Apparently none of these investigators realized that the phenomena observed in forced breathing are identical with those seen in tetany, and no effort was made to determine the factors upon which they depend.

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# A STUDY OF THE CARBOHYDRATE TOLERANCE IN ECK FISTULA AND HYPOPHYSECTOMIZED ANIMALS (POSTERIOR LOBE REMOVAL)

## LIVER FUNCTION IN THE METABOLISM OF SUGARS

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In the course of some studies in the Hunterian Laboratory on the sugar tolerance of animals deprived of the hypophyseal gland (1) it was shown that there was a temporary lowering of the assimilation limit for saccharose (the sugar which was primarily used for reasons then given), often with spontaneous mellituria—usually dextro-rotatory, occasionally laevo-rotatory—followed by a subsequent rise to above normal. It was observed at the same time that this artificially raised tolerance as well as the normal tolerance of animals (cat, dog and rabbit) could be lowered occasionally to the point of spontaneous glycosuria by the administration, intravenously or by mouth, of hypophyseal posterior lobe extracts. It was assumed and has been since proven that the administration of the extract had a glycogenolytic effect and was accompanied by a hyperglycemia. In the further investigation of this subject, some efforts were made to determine the source of this glycogen. This was first attempted by staining methods—Best's Carmine of the liver—before and after discharge of the glycogen either by Bernard's Piqure, by stimulation of the hypophysis or by giving hypophyseal or adrenal extracts. Though a lessening in the glycogen content of the cells could be demonstrated, the cells were never found entirely free from glycogen and the method had to be abandoned. Marked fatty changes in the liver had also been noted in some of the hypophysectomized animals and the suggestion arose that these changes might produce modifications in the storage capacity of the liver for glycogen and for carbohydrate metabolism. It was therefore decided to use an Eck fistula animal and to note what effect the shunting of the

liver from the portal circulation would have upon the sugar tolerance both before and after hypophysectomy.

The Eck fistula operation was first successfully performed on animals by von Eck in 1877 (2) and repeated with equal success by Stolnikow in 1882 (3). It consisted essentially of making an anastomosis of the portal vein to the vena cava with subsequent ligation and more recently, double ligation and transection of the portal vein near the liver hilus. In such a condition the blood supply of the liver is maintained by the intact hepatic artery, but the blood coming from spleen, pancreas, stomach and intestines, carrying with it the products of gastro-intestinal and splenic activity, is carried into the vena cava instead of to the liver and thus directly into the venous circulation. The purpose of the original operation was a clinical one, that of furnishing drainage for the abdominal fluid, ascites, incident to a cirrhosis of the liver, and neither of these two original investigators did any reliable work on changes in the metabolism. The operation has never proven successful when applied to human subjects both because of the operative difficulties and of the danger of subsequent toxic effects. It has, however, on repeated occasions been of extreme value in physiological investigations regarding carbohydrate metabolism and liver function.

In 1893 Hahn, Massen, Nencki and Pawlow (4), working with Eck fistula dogs, showed that such animals fed on a meat diet developed marked toxic effects, usually ending in the death of the animal. There was noted a definite decrease in the urinary output of uric acid and ammonia and a large increase in carbamate. On injecting the latter substance into normal and Eck fistula animals, they were able to produce similar toxic effects and concluded that accumulation of carbamate was the causal factor of the toxic symptoms. Rothberger and Winterberg in 1895 (5) verified the work of Pawlow *et al*, but could not satisfy themselves that carbamate was the toxic agent, concluding indefinitely that the toxic effect was caused by some substance which was removed by the liver under normal conditions but which, under the altered conditions of Eck fistula, accumulated in the blood. Nencki, Pawlow and Zaleski in 1896 (6) from experimental evidence concluded that ammonia was the cause of the toxic symptoms and not the carbamate. However in 1897 Nencki and Pawlow (7), by further experimentation with Eck fistula animals in which large portions of the liver were either removed or thrown out of function by ligation of the hepatic artery, concluded that the extreme toxic symptoms were far out of proportion to the increase of ammonia and could not consider the latter

as the main causal factor. Popelski in 1898 (8) showed that the Eck fistula dog had a low carbohydrate tolerance and that an alimentary glycosuria resulted when such an animal was fed large quantities of glucose. Sachs in 1898 (9), working with frogs whose livers had been extirpated, showed that they had a lower tolerance for laevulose than normal intact control animals, a finding which could not be confirmed with dextrose, galactose or arabinose. In 1901 Strauss (10), working with Sachs's findings as a rationale, introduced the laevulose test for hepatic insufficiency. In 1902 Schultz and Miller (11) had the opportunity of closely observing a clinical case of portal embolus at the liver hilus and were unable to find any definite untoward symptoms except that of ascites, reporting no pathological findings in urine. In 1907 Filippi (12) showed that there was a lowered tolerance for carbohydrates in dogs subsequent to Eck fistula operation. He showed that such animals possess an increased muscle glycogen content characteristic of over-nutrition and a low liver glycogen content characteristic of inanition. He inferred that the muscles could form glycogen independently and that the liver was neither specific for nor indispensable for carbohydrate metabolism. Hawk in 1908 (13), from feeding experiments, showed that quite extreme toxic effects were produced by feeding meat extracts (especially Liebig's extracts of meat) in conjunction with the meat diet. He was, however, unable to produce any such toxic symptoms by the introduction of carbamate nor was he able to produce glycosuria on carbohydrate feeding. Fischler in 1910-11 (14) noted the acute degeneration in the liver and fat necrosis following Eck fistula such as could be produced by pancreatic injury. These could be prevented by preliminary injections of trypsin. The meat intoxication, according to his view, was caused by an alkalosis due most probably to ammonia unneutralized by the liver and this condition could be relieved by acids. Michaud in 1911 (15) showed that Eck fistula prevented adrenal glycosuria but that when such an animal is fed sugar by mouth, the blood sugar either remains normal or increases within normal limits, as in any intact animal. In 1911 Voegtlin and Bernheim (16) showed that the bile pigments and bile acids were decreased in an Eck fistula dog to such an extent that the ligation of the common duct was not followed by jaundice and by only a trace of bile in the urine. They noted also the occasional attacks of convulsions, somewhat similar to those occurring in tetany, produced by meat feeding and showed that these could be prevented by feeding plenty of calcium in the form of bone. They inferred that the Eck fistula condition was compatible

with life if care was used in feeding. In 1911 Towles and Voegtlin (17) showed that there was no essential difference in the metabolism of creatin and creatinin in a normal and an Eck fistula dog and concluded that the liver is not an organ of prime importance in the metabolism of these substances and that the creatinin could hardly be held accountable for the toxic symptoms evoked on meat feeding. In thus excluding the portal circulation from the liver and diverting the portal stream directly into the systemic venous circulation, there is seen to be a reduction in the liver function: *a*, in the synthesis of the urea from ammonia salts; *b*, in the production of bile salts and bile acids; and *c*, in the lowered carbohydrate tolerance. It is with this latter feature that this paper is mainly concerned.

*Conduct of experiments.* Normal healthy dogs were used throughout the experiment—good-sized animals and those only who proved adaptable to sugar feeding on the pre-operative tolerance determinations. To have comparable results, weighed quantities from the same lot of sugar were used on all the animals. Sugar feedings were under as uniform conditions as possible,—namely, one sugar feeding a day, on empty stomach, 4 to 5 hours after meals, with definite amounts of water for dissolving the sugar and avoidance of all food or drugs with the feedings. Sugar in the urine was tested for and determined by Fehling, Nylander, fermentation and the polariscopic methods. The animals were fed on a mixed diet of boiled meat and bread, and were kept in cages during the tests. The urine was collected from beneath the cages.

The Eck fistula operation consisted of making a typical lateral anastomosis between the portal vein and the inferior vena cava using small enterostomy clamps (Jerusalem-Jeger) and silk for the sutures. On completion of the anastomosis, the portal vein was doubly ligated and transected between the ligatures, near the liver hilus.<sup>1</sup> Posterior

<sup>1</sup> Following Eck fistula operation, the animals remained apparently well and in all outward respects normal. There is an early but decreasing tendency to loss of weight and at first bounteous feedings are necessary to maintain the general body weight. When fed on a meat diet for a great length of time or by the addition of meat extract to a diet for several days, convulsive attacks somewhat suggestive of tetany are likely to occur. These are characterized by disorientation, indifference to surroundings, muscular twitchings, paralysis of the limbs, rigidity of back, ataxia and fine tremors. These may last as long as 48 hours and may lead to the death of the animal. The attacks are usually preceded by a period during which the animal seems below par, may be dull and drowsy or may be unusually restless or nervous. At this stage the acute attack may occasionally



lobe hypophysectomy was performed by the intracranial method described by Crowe, Cushing and Homans (18). This consists of contralateral temporal openings through bone and dura which allows the brain to be lifted and dislocated upwards through one decompression opening. The posterior lobe is then enucleated from the dangling gland from below, through the temporal opening of the opposite side.

### *Protocols*

*Animal A.* Well-nourished, normal male dog, weight 11.2 kilos

April 10-16. Pre-operative cane sugar tolerance, 130-140 grams

April 17-28. Eck fistula operation—uneventful convalescence

April 28-May 15. *Post-operative sugar tolerance. Weight 10.8 kilos*

Cane sugar, 14 grams. Laevo sugar in urine

Glucose, 55-60 grams

Laevulose, 7-8 grams

May 25-June 3. *2nd post-operative sugar tolerance*

Cane sugar, 14-25 grams

Glucose, 55-60 grams

Laevulose, 7-8 grams

June 18-25. *3rd post-operative sugar tolerance. Weight 10.8 kilos*

Cane sugar, 14-15 grams

Glucose, 55-60 grams

Laevulose, 7-8 grams. Mild convulsive attacks

July 7-15. *4th post-operative sugar tolerance. Weight 10.2 kilos*

Cane sugar, 14-15 grams

Glucose, 55-60 grams

Laevulose, 7-8 grams

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be aborted by feeding the animal plenty of bone, a substance for which he seems particularly desirous. Injections of calcium chloride or feedings of calcium lactate do not seem to have any effect when the acute attacks have already begun. After the attack has worn away the animal seems to be in normal condition again. One of the animals died during a severe second attack while the other two dogs had two and five attacks respectively. If care is taken to keep the animal on a mixed diet and excess of meat is avoided and if there is plenty of calcium in the form of bone, there seems to be no reason why an Eck fistula animal should not live for a long period of time. The animals reported here were observed for 139, 340 and 408 days respectively after the Eck fistula operation; the two latter finally succumbed to conditions unassociated with the fistula operation.

On autopsy the only pathological findings of interest are those associated with the liver. This organ is small, hard, firm and rather tougher than normal. The gall bladder and bile ducts appear to be normal. On section, the liver lobules seem to be decidedly smaller than normal with central atrophy and some fatty degeneration, conditions which have been produced experimentally by a reduction of the blood supply of the liver itself.

- July 17-30. Hypophysectomy—posterior lobe removal—ether  
 Urinary output from 1520 cc. to normal in 5 days. No permanent polyuria  
 Slight trace of sugar in the first specimen; no permanent glycosuria
- August 3-10. *1st post-operative tolerance. Weight 11.2 kilos*  
 Cane sugar, 14-15 grams  
 Glucose, 45-50 grams  
 Laevulose, 7-8 grams
- August 18-24. *2nd post-operative tolerance*  
 Cane sugar, 14-15 grams  
 Glucose, 50-55 grams  
 Laevulose, 7-8 grams
- September 6-13. *3rd post-operative tolerance. Weight 11.3 kilos*  
 Cane sugar, 14-15 grams  
 Glucose, 55-60 grams  
 Laevulose, 7-8 grams
- September 20-28. *4th post-operative tolerance*  
 Cane sugar, 14-15 grams  
 Glucose, 60-70 grams  
 Laevulose, 7-8 grams
- October 5-13. *5th post-operative tolerance. Weight 10.8 kilos*  
 Cane sugar, 14-15 grams  
 Glucose, 65-70 grams  
 Laevulose, 7-8 grams
- October 20-28. *6th post-operative tolerance*  
 Cane sugar, 14-15 grams  
 Glucose, 65-70 grams  
 Laevulose, 7-8 grams
- November 6-15. *7th post-operative tolerance*  
 Cane sugar, 14-15 grams  
 Glucose, 65-70 grams  
 Laevulose, 7-8 grams. Mild convulsions
- November 24-30. *8th post-operative tolerance. Weight 10.8 kilos*  
 Cane sugar, 14-15 grams  
 Glucose, 65-70 grams  
 Laevulose, 7-8 grams

*Summary of animal A*

	NORMAL TOLERANCE	AFTER ECK FISTULA	AFTER HYPO- PHYSECTOMY
	grams	grams	grams
Cane sugar.....	130-140	14-15	14-15
Glucose.....		55-60	65-70
Laevulose.....		7-8	7-8

Animal used for pancreatectomy—died May 24 of pneumonia.

In this, the first of the Eck fistula animals in good condition, the saccharose tests even in small amounts gave sugar which proved to be laevo-rotatory. As the saccharose is necessarily broken up into dextrose and laevulose in the process of its absorption and utilization in the body, it seemed necessary to resort to separate tests for the tolerance of the two sugars before the operation. It showed also that the assimilation tests under the circumstances with a complex sugar like saccharose, such as had been used, was capable of misinterpretation; for with hepatic insufficiency there would always be a low assimilation limit and a laevo-rotatory substance in the urine whereas the glucose tolerance itself might be little modified.

*Animal B.* Well-nourished, normal male dog, weight 13.3 kilos

May 11-June 6. *Pre-operative sugar tolerance. Weight 13.3 kilos*

Cane sugar, 170-175 grams

Glucose, 110-115 grams

Laevulose, 85-90 grams

June 6-19. Eck fistula operation—uneventful convalescence

June 19-July 10. *Post-operative sugar tolerance*

Cane sugar, 10-15 grams

Laevulose, 7-8 grams

Glucose, 75-80 grams.

July 12-22. *Hypophysectomy—posterior lobe removal—ether. Weight 12.3 kilos*

Urinary output 1360 cc. to normal in 7 days. No permanent polyuria

No glycosuria

July 23-August 2. *1st post-operative tolerance*

Laevulose, 8-9 grams

Cane sugar, 10-15 grams

Glucose, 70-80 grams

August 10-18. *2nd post-operative tolerance. Weight 12.6 kilos*

Laevulose, 8-9 grams

Cane sugar, 10-15 grams

Glucose, 70-80 grams

August 28-September 8. *3rd post-operative tolerance*

Laevulose, 8-9 grams

Cane sugar, 10-15 grams

Glucose, 100-110 grams

September 17-24. *4th post-operative tolerance. Weight 12.7 kilos*

Laevulose, 8-9 grams. 1st series of convulsions

Cane sugar, 10-15 grams

Glucose, 100-110 grams

October 4-10. *5th post-operative tolerance*

Laevulose, 8-9 grams

Cane sugar, 10-15 grams

Glucose, 100-110 grams

October 17-22. *6th post-operative tolerance. Weight 12.9 kilos*

Laevulose, 8-9 grams

Cane sugar, 10-15 grams

Glucose, 100-110 grams. 2nd convulsive attack

Animal died on October 24—2nd series of convulsions

*Summary of animal B*

	NORMAL TOLERANCE	AFTER ECK FISTULA	AFTER HYPO- PHYSECTOMY
	grams	grams	grams
Cane sugar.....	170-175	10-15	10-15
Glucose.....	110-115	75-80	100-110
Laevulose.....	85-90	8-9	8-9

*Animal C.* Well-nourished, normal male dog, weight 14.6 kilos

October 12-November 1. *Pre-operative sugar tolerance*

Cane sugar, 170-175 grams

Glucose, 120 grams

Laevulose, 90 grams. *Weight 16 kilos*

November 2-10. Eck fistula operation—uneventful convalescence

November 10-December 1. *Post-operative sugar tolerance*

Cane sugar, 20-25 grams

Laevulose, 7-8 grams

Glucose, 100 grams. *Weight 12.7 kilos*

December 2-12. *Hypophysectomy*—posterior lobe removal—ether

Urinary output 1970 cc. to normal in 10 days. No permanent polyuria

No glycosuria

December 12-January 1. *1st post-operative tolerance*

Laevulose, 7-8 grams

Glucose, 80-90 grams

Cane sugar, 20-25 grams. *Weight 12.7 kilos*

January 12-19. *2nd post-operative tolerance*

Laevulose, 7-8 grams

Glucose, 80-90 grams

Cane sugar, 20-25 grams. 1st attack of convulsions

January 29-February 6. *3rd post-operative tolerance*

Laevulose, 7-8 grams

Cane sugar, 20-25 grams

Glucose, 80-90 grams. *Weight 12.9 kilos.* 2nd attack of convulsions

February 17-24. *4th post-operative tolerance*

Laevulose, 7-8 grams

Cane sugar, 20-25 grams

Glucose, 80-90 grams

March 14-23. *5th post-operative tolerance*

Laevulose, 7-8 grams

Cane sugar, 20-25 grams

Glucose, 90-100 grams. *Weight 13 kilos*

- April 9-20. *6th post-operative tolerance*  
 Laevulose, 7-8 grams  
 Cane sugar, 20-25 grams  
 Glucose, 110-120 grams. 3rd attack of convulsions
- May 15-26. *7th post-operative tolerance*  
 Laevulose, 7-8 grams  
 Cane sugar, 20-25 grams  
 Glucose, 140-150 grams
- June 8-14. *8th post-operative tolerance*  
 Laevulose, 7-8 grams  
 Cane sugar, 20-25 grams  
 Glucose, 140-150 grams. *Weight 13.1 kilos*
- July 6-14. *9th post-operative tolerance*  
 Laevulose, 7-8 grams  
 Cane sugar, 20-25 grams  
 Glucose, 140-150 grams. 4th attack of convulsions
- July 28-August 4. *10th post-operative tolerance*  
 Laevulose, 7-8 grams  
 Cane sugar, 20-25 grams  
 Glucose, 140-150 grams. *Weight 13.3 kilos*
- August 11-16. *11th post-operative tolerance*  
 Laevulose, 7-8 grams  
 Cane sugar, 20-25 grams  
 Glucose, 140-150 grams
- August 24-30. *12th post-operative tolerance*  
 Laevulose, 7-8 grams  
 Cane sugar, 20-25 grams  
 Glucose, 140-150 grams. *Weight 13.3 kilos*

*Summary of animal C*

	NORMAL TOLERANCE	AFTER ECK FISTULA	AFTER HYPO- PHYSECTOMY
	grams	grams	grams
Cane sugar.....	170-175	20-25	20-25
Glucose.....	120-130	90-100	140-150
Laevulose.....	90-95	7-8	7-8

Animal used for pancreatectomy—died December 15.

The tolerance of an animal for sugar is the lowest dose of that sugar, following the ingestion of which, sugar just appears in the urine—the threshold dose producing glycosuria. It is dependent upon a number of factors such as the variety of sugar, the general state and condition of the animal, the manner and method of administering the sugar. This threshold dose is an indefinite amount varying between rather wide limits. Of the several methods of introducing sugar,—intra-

venous, subcutaneous and alimentary,—the two former give the lower values and the latter, the most frequently used, the highest values. For alimentary glycosuria the absorption rate from the gastro-intestinal tract must exceed the storage and utilization power of the tissues so that a hyperglycemia is produced with a blood sugar content high enough to overcome the normal impermeability of the kidney. It is evident that anything that concerns the speed of absorption, the utilization rate, the storage power of the tissue and the permeability of the kidney will result in changes in the tolerance. It is also evident that small amounts of sugar, through rapid absorption, will produce an abrupt rise in the blood sugar content with resultant glycosuria while larger amounts with slower absorption may be unaccompanied by the appearance of the sugar in the urine. The rate of absorption of sugar from the gastro-intestinal tract is not a constant factor and, as such, influences markedly the amount of sugar brought to the blood and tissues for utilization. The higher the tolerance, the more inaccurate the determination is and the greater the limits of variance. With the lower tolerance sugars, the determination is less difficult and the limits more readily defined. In many cases it is extremely difficult, and in some animals impossible, to obtain the tolerance with high tolerance sugars on account of the intolerance of stomach and intestinal tract for the huge and abnormal masses required for one feeding. The tolerance is usually stated in grams per kilo of body weight. This is open to grave error as young animals have a much lower tolerance per kilo than adult animals and obesity, as is well known, is not accompanied by a proportionate increase in the sugar tolerance. The indefiniteness of alimentary sugar tolerances is readily seen when one peruses the literature for tolerances determined by different investigators. Hofmeister's (19) tolerances for glucose in dogs ranged from 1.3 to 5.8 grams with 2 to 3 grams per kilo as the average. This is an unusually low tolerance, in fact much lower than that after intravenous administration and is rather out of the question. Pflueger (20) found some 11 to 16 grams per kilo. That the method of administration had a definite effect upon the tolerance is seen by his figures of 11 to 12 grams when the dextrose was given with meat stew and of less than 8 grams per kilo when it was given with soup. Schlessinger (21) found the tolerance to be about 10 to 11 grams per kilo and that small young animals had a lower tolerance per kilo than the larger adult animals. Boeri and De Andreis (22) found values of 4 to 6 grams for fasting animals and 10 to 13 grams for well-nourished animals. Pratt and

Spooner (23) found values of 11.5 grams for dogs. Quarta (24) found an average of 4 grams for male and 10 grams for female dogs. Sex has been found to have no appreciable effect upon the sugar tolerance and his value of 4 grams per kilo is entirely too low. Filippi's values were 8 to 10 grams for fasting animals and he lays great stress upon the fact that to obtain comparable results in alimentary feeding, sugar should be given under certain definite conditions. The tolerance values given for laevulose are few in number. Quarta gave the value as between 3 to 4 grams per kilo and de Filippi as about 1.5 gram. The tolerance for saccharose shows the same wide variation. Hoepppe-Seyler (25) sets it at 20 to 30 grams per kilo, Hofmeister at 3.6 grams and de Filippi at about 4 grams per kilo.

The osmotic irritation, the gastric and intestinal intolerance for large and abnormal masses of sugar, the lack of any control over the varying rates of absorption, make the alimentary method, though practically the only feasible one, far from an ideal one for investigative purposes. With an adaptable animal, under uniform conditions, comparative results on the same animal may however furnish data of some value.

Table 1 gives the tolerances obtained in this investigation. The normal values differ considerably from those given by de Filippi. The saccharose tolerances agree fairly closely with those obtained by Goetsch, Cushing and Jacobson who found in their series of animals weighing 9 to 11 kilos, a tolerance of 9 to 13 grams per kilo of body weight.

*Tolerance after Eck fistula operation.* Following Eck fistula operation there are marked changes in the carbohydrate tolerance ascertained by alimentary feeding. Subsequent to this surgical procedure there is usually a marked loss in body weight, and some of the post-operative tolerances given in the above table are undoubtedly high since they are calculated upon the lowered body weight. The glucose tolerance is modified to a less degree than the laevulose or cane sugar tolerance. This seems to indicate that the muscle and the body tissue generally can manage very well the storage and utilization of the glucose that is shunted to them by the Eck fistula operation and leads one to infer that the liver is an organ not absolutely necessary for the utilization of that sugar.

The most marked changes occur in the laevulose tolerance. In an Eck fistula animal a small amount of laevulose ingested is followed by a laevulosuria, there being almost an intolerance for that particular sugar. Muscular tissue can undoubtedly utilize a small amount of

laevulose as is evidenced by muscle perfusion experiments. It is questionable whether the muscles can change laevulose into glycogen and to any large extent thus utilize it. It seems quite probable that the liver acts as an organ of storage for the laevulose and alters it in some manner or other to make it more utilizable by the tissues. The liver evidently is indispensable for laevulose metabolism.

TABLE 1  
*Sugar tolerance; grams per kilogram of body weight*

	NORMAL	AFTER ECK FISTULA	AFTER HYPOPHYSECTOMY
<i>Dog A</i>			
Weight.....	11.4 kilos	11.4-10.2 kilos	10.8 kilos
Cane sugar.....	12.0 grams	1.4 grams	1.3 grams
Glucose.....		5.6 grams	6.2 grams
Laevulose.....		0.7 gram	0.7 gram
<i>Dog B</i>			
Weight.....	13.3 kilos	13.3-12.3 kilos	12.3-12.9 kilos
Cane sugar.....	13.3 grams	0.9 gram	1.0 gram
Glucose.....	8.4 grams	5.9 grams	8.0 grams
Laevulose.....	6.5 grams	0.6 gram	0.6 gram
<i>Dog C</i>			
Weight.....	14.6 kilos	16.0-12.5 kilos	12.7-13.3 kilos
Cane sugar.....	11.7 grams	2.0 grams	1.7 gram*
Glucose.....	8.2 grams	8.0 grams	11.9 grams
Laevulose.....	6.2 grams	0.6 grams	0.6 gram

\* As this animal had lost considerable weight after the Eck fistula operation these values figured on the body weight are as a consequence rather high.

The tolerance changes shown by saccharose are similar to those shown by laevulose. This sugar is broken down into dextrose and laevulose in the process of assimilation and the split products exert their respective effects upon the tolerance. Small doses of saccharose given to an Eck fistula animal are followed by a laevulosuria.

These changes in tolerance subsequent to Eck fistula operation in the dog, though the absolute tolerance values are quite different, are similar to those reported by de Filippi and are given here as confirmation of his work.



*Tolerance changes following subsequent hypophysectomy.* Following hypophysectomy in an Eck fistula animal the following changes were noted:

1. *Transient glycosuria.* In only one of the three cases was there a glycosuria following the operation. This was of a temporary character and appeared only in the first specimen of urine voided. There was no persistent or permanent glycosuria. Such a slight transient glycosuria was also found in three out of ten cases reported by Goetsch, Cushing and Jacobson, in their series of posterior lobe hypophysectomy. It is questionable whether this slight occasional glycosuria is due to the removal of the gland or incidental to the operation itself.

2. *Polyuria.* A definite post-operative polyuria was noted in all the three cases. Following operation there was a high urinary output of 1560, 1360 and 1970 cc. per day, gradually decreasing in amount so that the normal pre-operative urinary output was reached in 5 to 7 and 8 days respectively. The fluid intake on these days was increased also in proportionate amounts. There was no permanent or persistent polyuria as the result of the posterior lobe removal.

3. *Increased carbohydrate tolerance.* There was a moderate but definite increase in the tolerance for glucose after posterior lobe removal in all of the three animals, while the cane sugar and laevulose values remained essentially unmodified at their low levels. This augmentation of glucose tolerance came on extremely late, however, when compared to the augmentation following hypophysectomy in normal animals. In the series of hypophysectomies described by Cushing, Goetsch and Jacobson there was a transient lowering of carbohydrate tolerance followed by augmentation of tolerance reaching its height or greatest value from 9 to 21 days after the posterior lobe removal. In animal A there is a post-operative lowering of tolerance for 51 days, augmentation of tolerance during 28 days, making 79 days in all before the high tolerance was reached. No change in tolerance was noted subsequently during 51 days of observation. In animal B there was no definite post-operative lowering of tolerance, the tolerance reaching its highest value 58 days after operation with no change for 44 days subsequent observation. Animal C showed a low post-operative tolerance for 111 days, augmentation during 84 days or 195 days before the high value was reached. There was no change in tolerance on 96 days of further observation. The conclusion is quite evident that this augmented tolerance, indicative of increased storage and utilization capacity for converted carbohydrates, is the result of some extra-hepatic

function. It is most probably associated with the glycogenic and glycolytic power of the muscle tissue itself. Whatever function or capacity the liver has in this direction is readily assumed by the muscles themselves when the liver is removed from the portal circulation.

#### CONCLUSIONS

1. Eck fistula animals have an extremely low tolerance for laevulose. The liver is evidently essential for laevulose metabolism. The function of converting laevulose into glycogen is possessed by the liver; this function is permanently lost when the portal blood is diverted into the vena cava by Eck fistula.

2. Glucose tolerance is only slightly modified in Eck fistula animals. The liver is not entirely essential for glucose metabolism. The muscles undoubtedly perform well the functions of glycogenesis and glycolysis when the liver is shunted out of the portal circulation.

3. The glycogenic capacity of muscle is increased following posterior lobe removal in an Eck fistula animal as in an intact animal. The augmentation of tolerance is, however, considerably slower. No augmentation of laevulose tolerance is noted.

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## THE GASTRIC RESPONSE TO FOODS<sup>1</sup>

### XII. THE RESPONSE OF THE HUMAN STOMACH TO PIES, CAKES AND PUDDINGS

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Among the preparations classed under the heads of pies, cakes and puddings are a great variety of food products, the majority of which are commonly used as desserts although, in this era of quick lunches, pies, doughnuts, cinnamon buns, etc., frequently usurp the position in the diet of more substantial foods. For this misuse there can only be the excuse of necessity. However, the pies, cakes and puddings include many highly nutritious and most pleasing foods for whose proper preparation the capable cook may well be praised.

In spite of their very wide use there is a general impression that pies, cakes and puddings as a class are rather indigestible. The blame for this may rest in part on the diversity of cooking, in part on the depressing effect on the appetite of these foods if very sweet and eaten between meals, and in part to their being frequently crammed into an already well-filled stomach.

Little study has been made of the response of the stomach to these classes of foods. Some experiments were made by Beaumont (1), although amounts were not controlled. This author found in his subject that rice left the stomach in 1 hour; sago, in 1½ hour; tapioca, in 2 hours; gelatin, in 2½ hours; sponge cake, in 2½ hours, and apple dumpling in 3 hours. Direct comparison with our results cannot be made; but, as an instance, where Beaumont found rice to leave in less than half the time required for gelatin we found the gelatin to leave sooner than any other pudding. Our average time for cake was also distinctly longer than found by Beaumont.

<sup>1</sup>The expense of this investigation was defrayed by contributions from Mrs. M. H. Henderson, The Curtis Publishing Company and Dr. L. M. Halsey.

Our experiments on gastric response were carried out according to a procedure previously described (2). The subjects of the test were normal medical students who were given the food preparations in lieu of breakfast about 8 o'clock in the morning. Residuums were not removed. About 90 experiments were carried out. The acid responses and evacuation times of 81 of these are charted in figures 1 to 28 and summarized in tables 1, 2 and 3.

#### RESPONSE OF THE HUMAN STOMACH TO PIES

*Fruit pies.* Among the fruit pies studied were apple, peach, cherry, pumpkin and raisin pies. Apple pie was tried out on four subjects (see figs. 1, 3, 4 and 6). This pie was evacuated in from 2 hours to  $2\frac{1}{2}$  hours, or an average emptying time of  $2\frac{1}{2}$  hours. Fairly high acidities were developed within an hour to an hour and a half, the highest value attained varying from 83 to 102, with an average of 94. Further, most of this acidity was due to free HCl, showing that these pies have but a slight combining power for acid, and that in spite of this high free acidity gastric evacuation was not delayed.

Peach pie was given to one subject (see fig. 1) who had previously received apple pie. The acid curves for the two pies were practically identical, an acidity of 98 being developed in an hour and a half with peach pie. This latter pie, however, left the stomach half an hour sooner than apple pie.

Cherry pie was tried out in two cases (see figs. 7 and 8). High acidities, due mainly to free HCl, were developed in both cases. The evacuation time of one subject was 3 hours as compared with  $2\frac{1}{2}$  hours for apple pie. Not quite so high an acidity was developed on cherry pie, perhaps because its higher sugar content depressed secretion slightly, an effect we have shown sugar solutions to produce (3).

Raisin pie was given to one subject (see fig. 10). It was evacuated quite rapidly (in  $2\frac{1}{2}$  hours) or in the same time as required for rhubarb pie, and more rapidly than mince or pumpkin pie. A moderate acidity (with little combined acid) was developed, as might be expected from a pie fairly high in sugar but containing little protein.

Rhubarb pie in general character and gastric response may be classed with the fruit pies. Two subjects were given this pie (see figs. 9 and 11). It was one of the most readily evacuated pies, leaving the stomach in the same time as raisin pie ( $2\frac{1}{2}$  hours) and more rapidly than cherry pie. The acid responses were high, averaging 101 at the maximum, nearly all of this being due to free HCl.

TABLE 1

*The response of the human stomach to pies*

NUMBER	KIND OF PREPARATION	TYPE OF INDIVIDUAL	EVACUA-TION TIME	HIGHEST TOTAL ACIDITY
1-Son	Pumpkin.....	Rapid	2:45	99.5
2-Wil	Pumpkin.....	Slow	2:00	102.5
3-Son	Mince.....	Rapid	2:45	107.5
4-Wil	Mince.....	Slow	3:15	105.0
5-Son	Lemon meringue.....	Rapid	2:30	74.0
6-Wil	Lemon meringue.....	Slow	2:30	95.0
7-Son	Raisin.....	Rapid	2:15	94.5
8-Rud	Cocoanut custard.....	Rapid	2:15	107.0
9-Rud	Custard pie.....	Rapid	2:30	84.5
10-Rud	Cherry.....	Rapid	2:45	99.0
11-Wil	Cherry.....	Slow	3:00	95.0
12-Mil	Peach.....	Rapid	2:00	98.5
13-Kar	Apple.....	Rapid	2:00	97.0
14-Lea	Apple.....	Rapid	2:30	83.0
15-Mil	Apple.....	Rapid	2:30	95.0
16-Wil	Apple.....	Slow	2:45	102.5
17-Rud	Rhubarb.....	Rapid	2:00	106.0
18-Son	Rhubarb.....	Rapid	2:15	95.0
Average.....			2:27	90.0
Pie combinations				
19-Rud	Cherry (with ice cream).....	Rapid	3:15	115.0
20-Lea	Apple (with ice cream).....	Rapid	2:30	69.5
21-Wil	Apple (with cheese).....	Slow	3:00	113.5
Average.....			2:55	99.0
Pie crust vs. insides				
22-Lea	Pie crust.....	Rapid	2:45	66.5
23-Mil	Pie crust.....	Rapid	2:45	92.5
24-Wil	Pie crust.....	Slow	3:15	102.5
25-Son	Pie crust.....	Rapid	2:45	98.5
26-Far	Insides (apple).....	Slow	3:15	93.0
27-Mil	Insides (peach).....	Rapid	1:30	103.5
28-Rud	Insides (rhubarb).....	Rapid	2:15	118.0
29-Wil	Insides (apple).....	Slow	2:45	99.0
Pie crust, average.....			2:52	90.0
Insides, average.....			2:26	103.0

Pumpkin pie differs somewhat in character from the fruit pies mentioned, but the acid response was not distinctly different. The acid combining power was slightly greater for pumpkin pie. A subject of

TABLE 2

*The response of the human stomach to cakes*

NUMBER	KIND OF PREPARATION	TYPE OF INDIVIDUAL	EVACUA-TION TIME	HIGHEST TOTAL ACIDITY
1-Far	Angels' food.....	Slow	4:30	97.5
2-Mur	Angels' food.....	Rapid	3:45	100.0
3-Owe	Angels' food.....	Rapid	2:45	99.0
4-Far	Devils' food.....	Slow	4:00	92.0
5-Mur	Devils' food.....	Rapid	3:00	83.0
6-Owe	Devils' food.....	Rapid	2:45	73.5
7-Gol	Fruit cake, fresh.....	Rapid	3:15	73.0
8-Mur	Fruit cake, fresh.....	Rapid	3:15	80.5
9-Gol	Fruit cake, old.....	Rapid	3:30	78.0
10-Mur	Fruit cake, old.....	Rapid	3:30	77.5
11-Mur	Lady fingers.....	Rapid	3:15	75.5
12-Cor	Crullers.....	Slow	4:15	88.0
13-Lot	Crullers.....	Rapid	2:15	91.5
14-Lot	Doughnuts.....	Rapid	2:30	83.0
15-Spe	Doughnuts.....	Slow	3:00	106.0
16-Cor	Cream puffs.....	Slow	2:30	107.0
17-Far	Ginger snaps.....	Slow	3:30	101.5
18-Gol	Spiced cookies.....	Rapid	2:15	89.0
19-Lot	Spiced cookies.....	Rapid	2:15	104.0
20-Owe	Chocolate layer cake.....	Rapid	2:30	28.5
21-Far	Mary Ann cookies.....	Slow	2:45	70.5
22-Mur	Cinnamon bun.....	Rapid	2:30	72.0
23-Spe	Cinnamon bun.....	Slow	2:45	127.5
24-Owe	Ginger bread.....	Rapid	2:15	65.0
25-Rud	Ginger bread.....	Rapid	3:00	106.0
26-Rud	Short cake, strawberry.....	Rapid	3:00	124.5
27-Ree	Short cake, strawberry.....	Slow	3:15	130.0
28-Spe	Bread with peanut butter.....	Slow	3:15	120.5
29-Owe	Bread with corn syrup.....	Rapid	2:15	55.0
Average.....			3:02	90.0

the slow emptying type required  $2\frac{1}{2}$  hours as compared with  $2\frac{1}{2}$  hours for raisin and rhubarb pies (see fig. 10). A subject of the rapid emptying type (see fig. 7) required only 2 hours for pumpkin pie as compared with 3 hours for cherry and  $2\frac{1}{2}$  hours for apple pie.

*Mince, custard and lemon meringue pies.* The mince, custard and meringue pies are higher in protein than simple fruit pies and might, therefore, be expected to undergo greater change in the stomach.

Mince pies are generally considered to be the least easily digested of pies, due to their content of meat and spices. This is borne out by our experiments (see figs. 7 and 10). Mince pie required  $3\frac{1}{4}$  hours to leave

TABLE 3  
*The response of the human stomach to puddings*

NUMBER	KIND OF PREPARATION	TYPE OF INDIVIDUAL	EVACUATION TIME	HIGHEST TOTAL ACIDITY
1-Dal	Indian . . . . .	Rapid	2:00	113.5
2-Gle	Corn starch . . . . .	Slow	2:30	92.5
3-Dal	Chocolate . . . . .	Rapid	2:00	110.0
4-Spe	Chocolate . . . . .	Slow	2:00	91.0
5-Dal	Brown Betty . . . . .	Rapid	2:15	69.5
6-Spe	Brown Betty . . . . .	Slow	2:45	110.0
7-Kar	Cabinet . . . . .	Rapid	2:00	87.0
8-Spe	Cabinet . . . . .	Slow	2:45	82.0
9-Lea	Rice . . . . .	Rapid	2:00	67.0
10-Mil	Rice . . . . .	Rapid	2:00	101.0
11-Lea	Rice with raisins . . . . .	Rapid	1:45	76.5
12-Mil	Cup custard . . . . .	Rapid	2:00	107.0
13-Gle	Cup custard . . . . .	Slow	4:15	112.0
14-Dal	Plum . . . . .	Rapid	2:15	113.0
15-Gle	Plum . . . . .	Slow	4:30	96.5
16-Kar	Bread . . . . .	Rapid	2:15	79.5
17-Dal	Bread . . . . .	Rapid	2:00	108.0
18-Gol	Bread . . . . .	Rapid	1:30	96.0
19-Kar	Bread . . . . .	Rapid	1:45	104.0
20-McD	Tapioca . . . . .	Slow	2:30	74.0
21-McD	Apple tapioca . . . . .	Slow	2:15	106.5
22-Col	Gelatine . . . . .	Rapid	2:00	61.5
23-Kar	Gelatine . . . . .	Rapid	1:30	62.0
Average . . . . .			2:18	92.0

the stomach of an individual who required  $2\frac{3}{4}$  hours for apple pie and 3 hours for cherry pie. The other subject required  $2\frac{3}{4}$  hours for mince and  $2\frac{1}{4}$  hours for either raisin or rhubarb pie. The highest acid response was given with mince pie, and the acid combining power was also markedly greater than for simple fruit pies.

One experiment each was made on plain custard and cocoanut custard pie, the same subject receiving both (see fig. 8). The plain custard



required 15 minutes longer to digest and developed a higher acidity due probably to the slightly higher protein content. The high combined acidities of both would be expected from their known content of milk and egg proteins. The custards required a longer digestion than rhubarb pie but were by no means handled with difficulty inasmuch as cherry pie took 15 minutes longer than either.

Related in composition to the custards are the lemon meringues which, when properly made, are fairly high in eggs. They leave the stomach in about the same time as the custards ( $2\frac{1}{2}$  hours) and show a less rapid development of acidity than other pies (figs. 7 and 10).

#### COMPARATIVE RESPONSE OF THE STOMACH TO PIE CRUST, CONTENTS AND WHOLE PIES

The popular opinion that pie crusts are the least digestible portions of ordinary pies would also appear probable from their known compositions, possessing as they do most of the protein and fat of many pies, especially fruit pies. The absolute digestibility of pie crusts must, of course, depend a good deal on their texture and composition. The response of the stomach to pie contents would be expected to approximate that of fruit sauces or puddings of similar composition. Thus we have found (4) that fruits in general leave the stomach very rapidly with the development of considerable free but little combined acidity.

Pie crust alone was given in 100-gram portions to four subjects (figs. 2, 5, 6 and 11). This was evacuated in from  $2\frac{3}{4}$  to  $3\frac{1}{4}$  hours. In the case of one subject the contents of a peach pie left the stomach in  $1\frac{1}{2}$  hour as compared with  $2\frac{3}{4}$  hours for the crust. For apple pie and crust the required periods were  $2\frac{3}{4}$  and  $3\frac{1}{4}$  hours respectively. Pie crust thus remains in the stomach distinctly longer than an equal weight of the contents of apple or peach pie. The acid responses of the two were not so different, although crust usually gave a slower rise with a more sustained curve.

The whole pie naturally is intermediate between pie crust and contents in the response it evokes. Thus subject "Mil" (figs. 1 and 2) retained peach pie 2 hours and contents  $1\frac{1}{2}$  hour. Another subject, "Rud" (fig. 9) required a few minutes longer for the rhubarb alone than for the pie, while a third subject, "Wil," took  $2\frac{3}{4}$  hours in each case (fig. 6). Thus these fruit pies require little longer to digest in the stomach than the interiors alone.

If crust is compared with whole pie, we find in the case of "Mil" the pies requiring 2 to  $2\frac{1}{2}$  hours and crust  $2\frac{3}{4}$  hours; in the case of "Son"  $2\frac{1}{4}$  hours for rhubarb pie and  $2\frac{3}{4}$  hours for crust alone. Subject "Wil" required  $2\frac{1}{2}$  to  $3\frac{1}{4}$  hours for pies (mince being the slowest) and  $3\frac{1}{4}$  hours for crust. Crust thus takes somewhat longer to digest in the stomach than the contents of all but the least digestible pies.

It will be seen, therefore, that the response of the stomach to whole pies approximates that of the pie contents, in spite of the fact that the crust alone requires a distinctly longer digestion period. We did not, however, find that crust was in any sense indigestible, although 100 grams of it were given at a time.

#### PIE WITH ICE CREAM AND WITH CHEESE

To the usual 100-gram portions of apple and cherry pie were added in each of two cases 50 grams of vanilla ice cream. The responses are charted in figures 4, 5 and 8. The cherry pie alone required  $2\frac{1}{4}$  hours. With ice cream it remained in the stomach half an hour longer. Apple pie, however, showed the same evacuation time ( $2\frac{1}{4}$  hours) in each case. In one case the total acidity was slightly higher after adding ice cream; in the other the reverse was true. It is clear, therefore, that ice cream, though greatly increasing the food value of the dish, need not necessarily increase the burden of the stomach to any marked extent.

Twenty grams of cream cheese were added to apple pie in one case and compared with apple pie alone (see fig. 6). It will be noted that the acid responses in the two cases do not differ greatly, although naturally cheese increases the acid combining power of the food ingested. The addition of the cheese delayed evacuation about a quarter of an hour, and led to a slight secretion of gastric juice after the stomach was otherwise empty.

#### THE RESPONSE OF THE HUMAN STOMACH } TO CAKES

Among the cakes and related products tested out by us were angels' food, devils' food, fruit cake, chocolate layer cake, lady fingers, strawberry short cake, crullers, doughnuts, cream puffs, cinnamon buns, ginger bread and cookies of different kinds. One hundred grams were given in each case.

*Angels' food, devils' food, fruit cake and short cake.* Angels' food was given to three men. A few days later an equal amount of devils' food was given to each of the same three subjects for comparison (see figs. 12, 13 and 15). It was found that angels' food cake remained on the average 20 minutes longer in the stomach than devils' food, the evacuation time of these cakes ranging from  $2\frac{1}{4}$  to  $4\frac{1}{4}$  hours. This may well be related to the greater protein content of the angels' food which is particularly high in eggs. The distinctly higher acidities developed in the stomach in the case of angels' food cake, and the much more pronounced acid combining power must also be due to the high protein content of this cake as compared with devils' food which, however, is higher in calories due to its sugar and chocolate content.

Chocolate layer cake (fig. 15) left the stomach of a subject of the rapid-emptying type in  $2\frac{1}{4}$  hours or a few minutes sooner than devils' food cake. The acidity developed was low, due perhaps in part to the depressing action of the sugar icing on secretion.

Old and fresh fruit cake were compared, using two subjects on each (see figs. 13, 14 and 17). It is a popular belief that old fruit cake is much more easily digested than the same cake in the fresh condition. Portions of freshly baked cake were given to the men, and three weeks later the same subjects were given other portions of the cake which had been kept in tin at room temperature for that period.

In each case it was found that while the fresh cake required  $3\frac{1}{4}$  hours for gastric digestion the older cake required  $3\frac{1}{4}$  hours. The acid responses were practically identical on the fresh and older cake. No distinct difference in gastric response to fresh and old fruit cake could, therefore, be detected.

Two subjects were given strawberry shortcake (75 grams cake and 25 grams of berries) without cream. This cake left the stomach in moderate time (3 to  $3\frac{1}{4}$  hours) and gave rise to the development of high intragastric acidities, in part probably due to the acidity of the berries themselves.

Lady fingers were given to one subject (see fig. 13). The evacuation time of these was  $3\frac{1}{4}$  hours as compared with 3 hours for devils' food and  $3\frac{1}{4}$  hours for fresh fruit cake.

*Doughnuts, crullers, ginger bread, cinnamon buns and cream puffs.* Ginger bread was given to two subjects (see figs. 9 and 16). It remained in the stomach from  $2\frac{1}{4}$  to 3 hours or a few minutes less than strawberry shortcake or chocolate layer cake and a shorter time than devils' food or angels' food cake. Ginger bread brought about a mod-

erate stimulation of acid secretion with, in one case, a rather slow development. Evidently the ginger did not induce any increased secretion of gastric juice.

Cinnamon buns were tested out on two subjects (figs. 14 and 21), and required from  $2\frac{1}{2}$  to  $2\frac{3}{4}$  hours. In one subject these buns left the stomach sooner than fruit cake, angels' or devils' food cake or lady fingers. The other subject evacuated cinnamon buns a few minutes sooner than doughnuts. In acid response the buns were found to follow the general trend of the cakes just mentioned, not attaining, however, to the level of angels' food cake. Neither is the acid response as high as that of bread with peanut butter. Probably the sugar of the buns depresses secretion somewhat.

Bread with peanut butter (100 grams bread and 25 grams of peanut butter) remained in the stomach  $3\frac{1}{4}$  hours as compared with  $2\frac{3}{4}$  hours for cinnamon buns. The acidity developed was considerably higher in the case of bread with peanut butter (see fig. 22).

Bread with corn syrup (80 grams of bread and 20 grams corn syrup) left the stomach in  $2\frac{1}{4}$  hours (fig. 16) or in the same time as ginger bread and a little sooner than chocolate layer cake or devils' food. The acidity developed was only moderate, being slightly lower than for ginger bread, the syrup probably depressing secretion.

Cream puffs were given to one subject (fig. 19). They developed an acidity of over 100 and left in  $2\frac{1}{2}$  hours or much sooner than crullers.

Crullers and doughnuts were compared on one subject (fig. 20). The doughnuts remained in the stomach 15 minutes longer than the crullers or  $2\frac{1}{2}$  hours. The maximum acidities developed were about the same in each case, but the acid development was slower in the case of crullers due, perhaps, to their higher sugar and fat content. One subject required  $4\frac{1}{4}$  hours for crullers. His digestion did not, however, appear to be entirely normal on the day of this test. A third subject required for doughnuts a digestion time of 3 hours as compared with  $2\frac{3}{4}$  hours for cinnamon buns (see fig. 21). It seems, therefore, that doughnuts are handled a little less readily than crullers, but that neither of these are unusually difficult for the normal stomach to handle.

*The response of the stomach to cookies.* Spiced cookies, ginger snaps and Mary Ann cookies were studied in a few cases. The Mary Ann cookies remained in the stomach of a subject of the slow-emptying type for  $2\frac{3}{4}$  hours as compared with 4 hours for devils' food and  $4\frac{1}{2}$  hours for angels' food cake, and this in spite of the higher content of the cookies in dry matter. Ginger snaps required in this subject  $\frac{3}{4}$  hour

longer than the Mary Anns. A high acidity was developed on ginger snaps, but this was very slow in development, indicating that the ginger depressed gastric secretion.

Spiced cookies left the stomach in  $2\frac{1}{2}$  hours in both cases where they were tried out. They remained the same time as crullers but not so long as doughnuts. They left an hour sooner than fruit cake. Fairly high acidities were developed by these cookies.

It appears, therefore, that cookies, in spite of their high content of dry matter, leave the stomach sooner than many cakes, and in really equivalent amounts would be handled more readily by the stomach. This may in part be due to their more granular texture.

#### THE DIGESTION OF PUDDINGS IN THE HUMAN STOMACH

The following puddings were studied: vanilla corn starch, chocolate corn starch, tapioca, apple tapioca, gelatin, Indian pudding, bread pudding, Brown Betty, cabinet, rice, rice with raisins, cup custard and plum pudding. One hundred grams of pudding were given in each case.

*Starch and tapioca puddings.* Five experiments were carried out on corn starch and tapioca puddings (see figs. 22, 23, 24 and 26). Vanilla and chocolate corn starch and plain tapioca and apple tapioca were tested. All of these puddings left the stomach in from 2 to  $2\frac{1}{2}$  hours. Apple tapioca was evacuated 15 minutes sooner than the plain pudding. It developed a higher acidity with less combined hydrochloric acid. The starch puddings left the stomach rather quickly and developed a moderately high acidity (91 to 110).

*Cereal puddings, cabinet, Brown Betty, bread pudding, rice pudding and Indian pudding.* Bread pudding quickly developed in the stomach a high total and moderate combined acidity and was rapidly digested, leaving the stomach in from  $1\frac{1}{2}$  to 2 hours in the case of subjects of the rapid-emptying type (see figs. 17, 24 and 27). Brown Betty pudding remained in the stomach  $2\frac{1}{2}$  hours as compared with 2 hours for bread pudding. A subject of the slow-emptying type required  $2\frac{1}{2}$  hours for this pudding (figs. 22 and 24).

Cabinet pudding left the stomach of a subject of the rapid type in 2 hours as compared with  $1\frac{1}{2}$  hours for bread pudding (see fig. 27). A subject of the slow type (see fig. 22) required  $2\frac{1}{2}$  hours or the same time as for Brown Betty. Cabinet developed a lower acidity than bread pudding.

Indian pudding gave a response practically identical with that of bread pudding, leaving the stomach in 2 hours and developing a high acid point of 113 at an hour and a half, the high protein content of this pudding being undoubtedly responsible for the high combined acidities found (see fig. 25).

Rice pudding was tried both with and without raisins (see figs. 1 and 4). The acid responses were in each case practically the same. The evacuation times on plain rice pudding were in each case 2 hours. Rice pudding with raisins required in one case 2 hours and in the other only 1½ hours. The combined acidities were practically the same in each case. The rice puddings are thus among the more easily digested puddings.

*Gelatin and plum puddings and cup custards.* A widely used gelatin dessert of strawberry flavor was given to each of two men (see figs. 27 and 28). It left their stomachs very rapidly (in 1½ to 2 hours) and gave rise to very little stimulation of acid secretion. The low combined acidities also indicate that this product leaves the stomach too soon and with too little stimulatory effect to be markedly altered in the stomach or to throw any considerable burden upon it.

Plum pudding was found to be one of the less readily digested puddings (see figs. 23 and 27). Its relatively high food value must, however, be borne in mind. A subject of the rapid-emptying type required 2½ hours to evacuate this food as compared with 1½ hours for gelatin and 1½ hours for bread pudding. A subject of slow-emptying type required 4½ hours for plum as compared with 2½ hours for corn starch pudding. Moderately high total and combined acidities were developed.

Cup custards remained in the stomach about the same time as Brown Betty pudding and a few minutes longer than bread pudding, corn starch or Indian puddings, but not so long as plum pudding (see figs. 23 and 24). Being very high in protein, custards would be expected to show a rapid gastric stimulation with much combined acidity. This was found to be the case.

#### COMPARATIVE RESPONSES OF THE STOMACH TO PIES, CAKES AND PUDDINGS

From tables 1, 2 and 3 the average acid responses and evacuation times of all subjects on pies, cakes and puddings may be obtained. They show that the grand average evacuation times were for puddings,

pies and cakes, 2:18, 2:27 and 3:02, respectively. The average highest total acidity was practically 90 in each of the three cases.

Perhaps a more significant comparison may be obtained from table 4, in which are summarized the results from six subjects, each of whom besides receiving a number of pies or cakes or puddings was also given certain foods belonging to one of the other two classes. It will be seen that here also pies left the stomach sooner than cakes, and puddings still more rapidly.

TABLE 4

SUBJECT	EVACUATION TIME AND ACIDITIES					
	Puddings		Pies		Cakes	
Gol.....	1:30	96.0			3:00	80.2
Spe.....	2:30	94.3			3:00	118.0
Kar.....	1:52	83.1	2:00	97.0		
Lea.....	1:67	71.5	2:30	76.0		
Mil.....	2:00	104.0	2:23	100.5		
Rud.....			2:41	101.4	3:00	115.0
Average.....	2:00	89.8	2:24	93.7	3:00	104.4

## SUMMARY AND CONCLUSIONS

A study was made of the acid responses and evacuation times of nearly fifty pies, cakes and puddings in the normal human stomach. The average evacuation time on puddings for all subjects was 2 hours and 18 minutes as against 2 hours and 27 minutes for pies and 3 hours and 2 minutes for cakes. Averaging the highest total acidities values were obtained for puddings of 92, for pies of 90 and for cakes of 90. Direct comparisons of the three types of foods on the same individuals indicated also that pies were handled more readily than cakes, and pudding somewhat more readily than either.

Fruit pies, such as apple, pumpkin, raisin and peach, left the stomach in from 2 to 2½ hours and developed a moderately high acidity (90 to 100). Most of the acidity is due to free HCl, the acid combining powers of these pies being low. Cherry pie, high in sugar, remained in the stomach a few minutes longer than the above. Rhubarb pie was treated in the same way as fruit pies, leaving the stomach in from 2 to 2½ hours.

Custard pies left the stomach in moderate time (2½ to 2¾ hours) and possessed a fairly high acid combining power. Lemon meringue

showed a similar response except that the acidity developed more slowly. Mince pies required a rather long time to leave the stomach (2½ to 3½ hours) and developed high total and combined acidities.

Pie crusts alone remained in the stomach distinctly longer than most whole pies or the contents alone of such pies, and gave a lower but more sustained acid curve. On the other hand, differences in evacuation time of whole pies and the contents of such pies were usually very slight so that pies with crust, if properly made, could by no means be classified as difficult for the stomach to handle.

The addition of 50 grams of ice cream to a small piece of pie did not increase the burden of the stomach to any marked extent. The addition of 20 grams of cheese to apple pie increased the digestion time only a few minutes.

Angels' food cake remained distinctly longer in the stomach than devils' food cake and developed a higher total and combined acidity.

Chocolate layer cake left the stomach in moderate time, acid secretion being depressed by the sugar of this cake.

Fresh and old fruit cakes showed almost identical acid responses and evacuation times in the human stomach.

Strawberry shortcakes left the stomach in moderate time (3 to 3½ hours) and developed high intragastric acidities.

Lady fingers left the stomach in 3 hours or in about the same time as other cakes.

Ginger bread evoked a rather slow acid response and left the stomach in moderate time.

Cinnamon buns left the stomach sooner than most cakes but with a similar acid response.

Bread with peanut butter remained in the stomach longer than cinnamon buns and developed a higher acidity.

Bread with corn syrup left the stomach in 2½ hours, the syrup depressing secretion somewhat.

Doughnuts remained in the stomach a few minutes longer than crullers. Acidities developed a little more slowly in the case of crullers, due perhaps to the sugar and fat content. These fried cakes required a digestion time but little longer than the average for cakes.

Cookies, in spite of their high content of dry matter, were found to leave the stomach sooner than most cakes, probably on account of their granular texture. Ginger cookies required a little longer than those less highly flavored, and the acid development was less rapid.



Chocolate corn starch pudding, rice pudding with or without raisins, Indian pudding, bread pudding and gelatin left the stomachs of individuals of the rapid-emptying type very quickly (in  $1\frac{1}{2}$  to 2 hours). Rice pudding with raisins left a little sooner than plain rice pudding.

Cabinet pudding, Brown Betty pudding, cup custard and apple tapioca required only a few minutes longer. Plain tapioca remained in the stomach a little longer than apple tapioca. Plum pudding left the stomach slowly.

The highest total and combined acidities were caused by Indian pudding, bread pudding and cup custard, all of these being high in protein. The other puddings developed moderate acidities except gelatin, which produced little acid stimulation and left the stomach very quickly.

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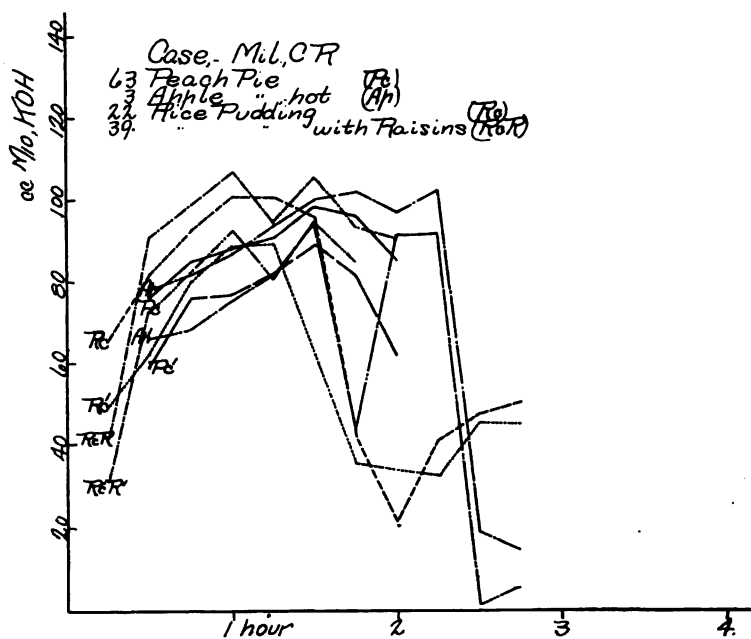


FIG. 1

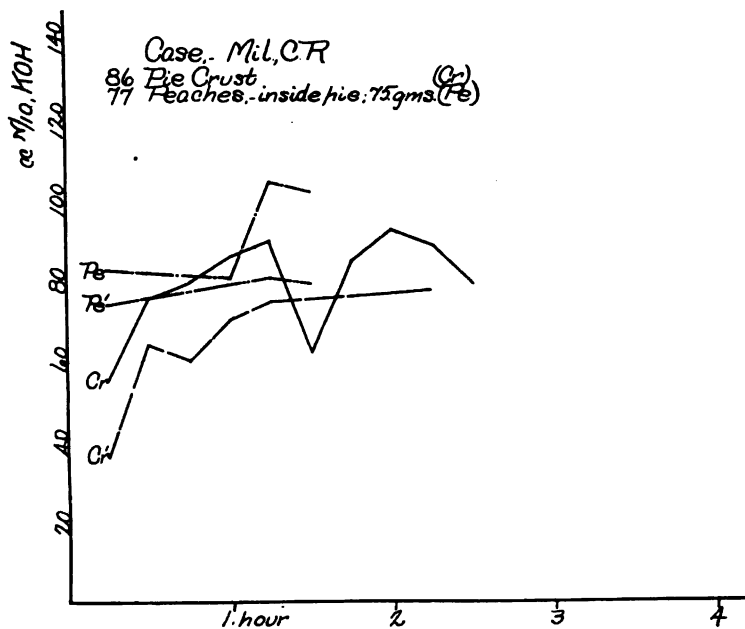
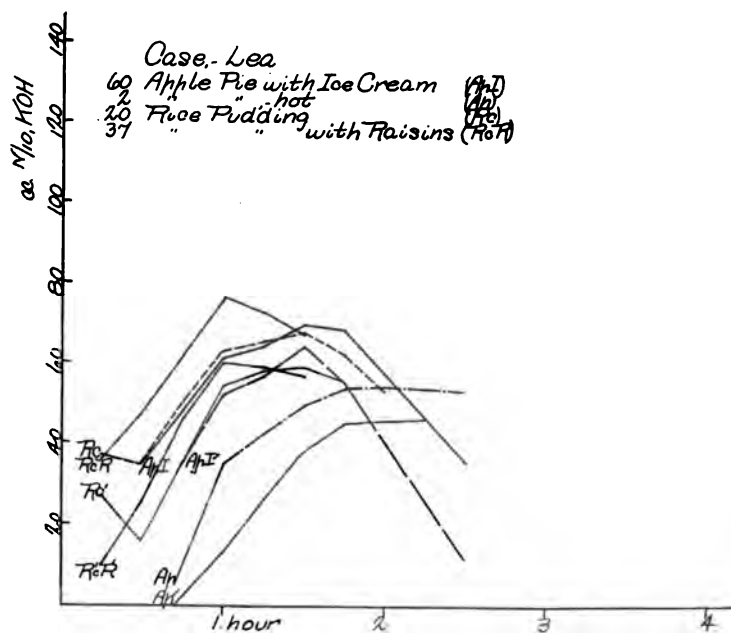
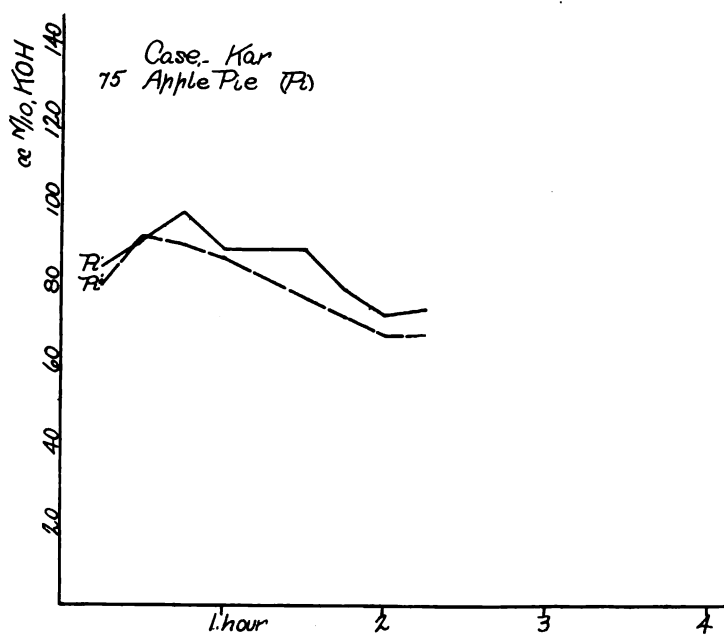


FIG. 2



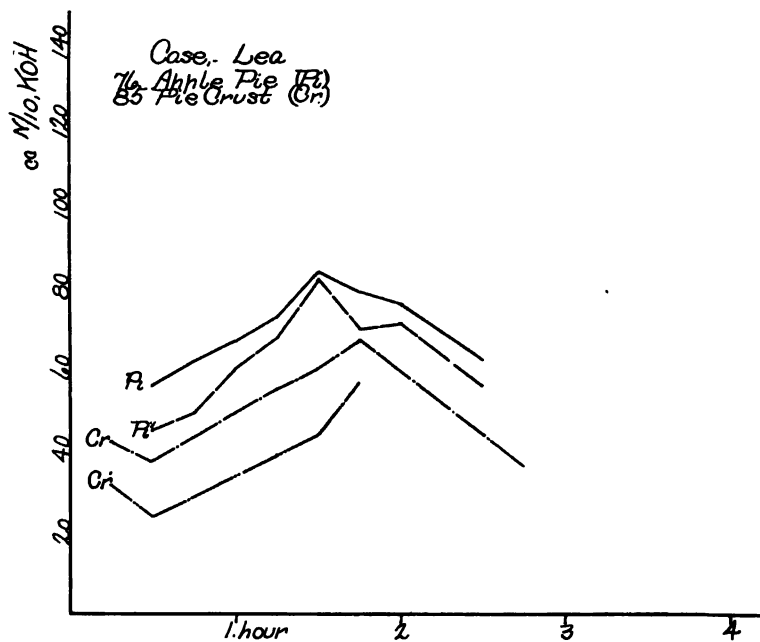


FIG. 5

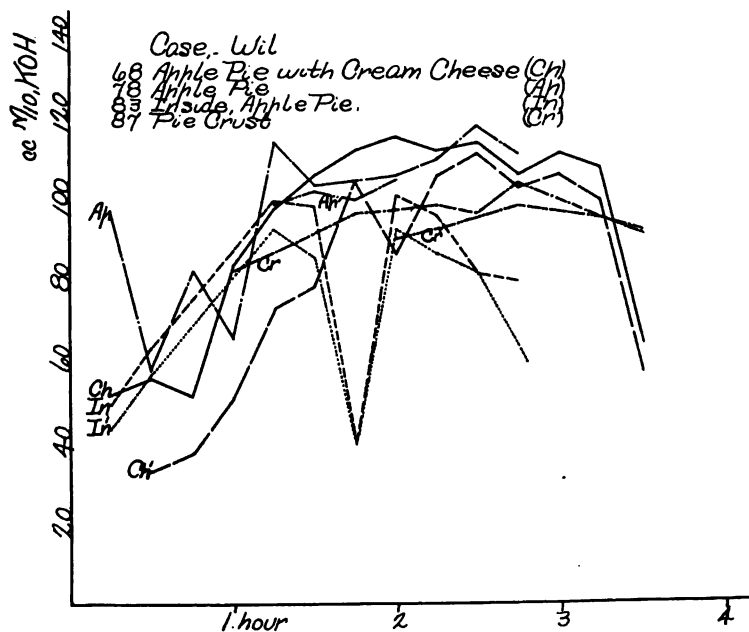


FIG. 6

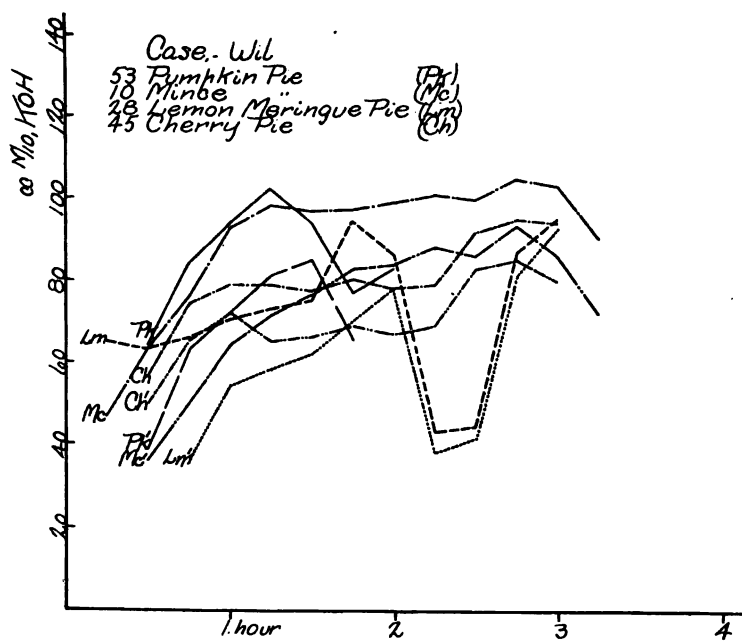


FIG. 7

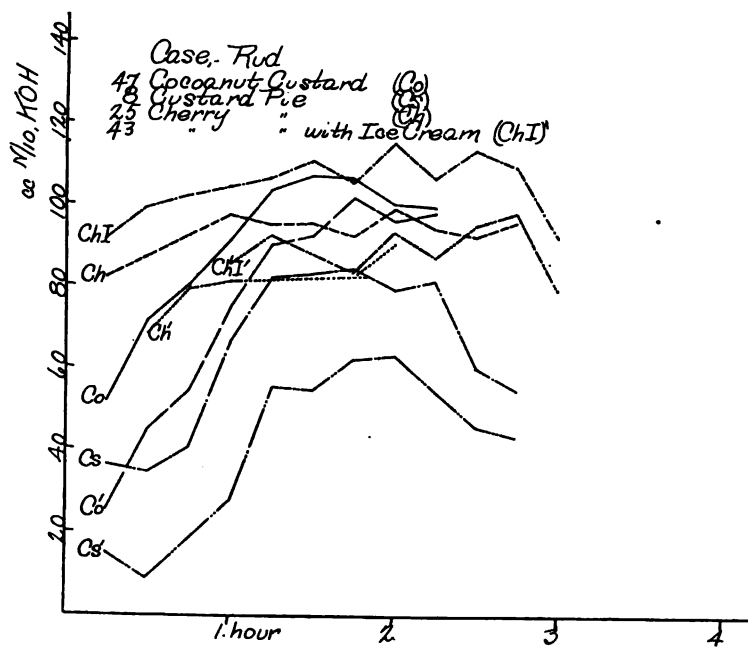


FIG. 8

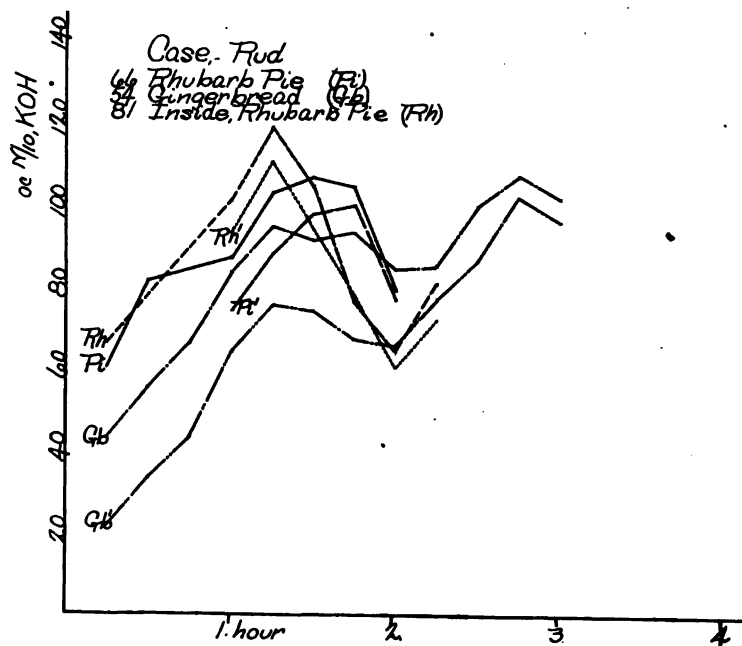


FIG. 9

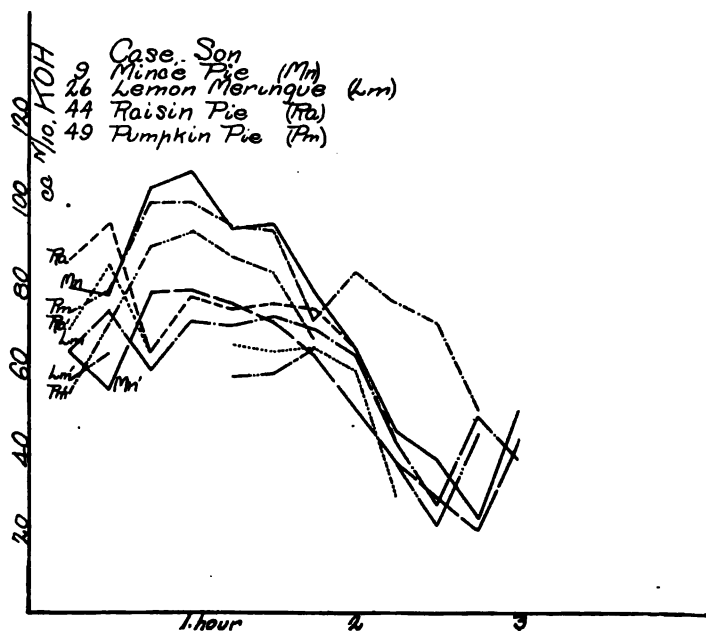


FIG. 10

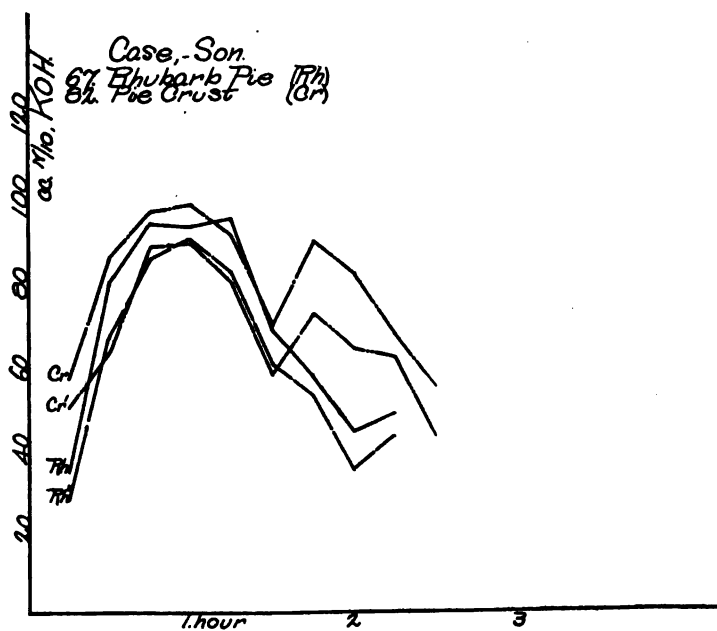


FIG. 11

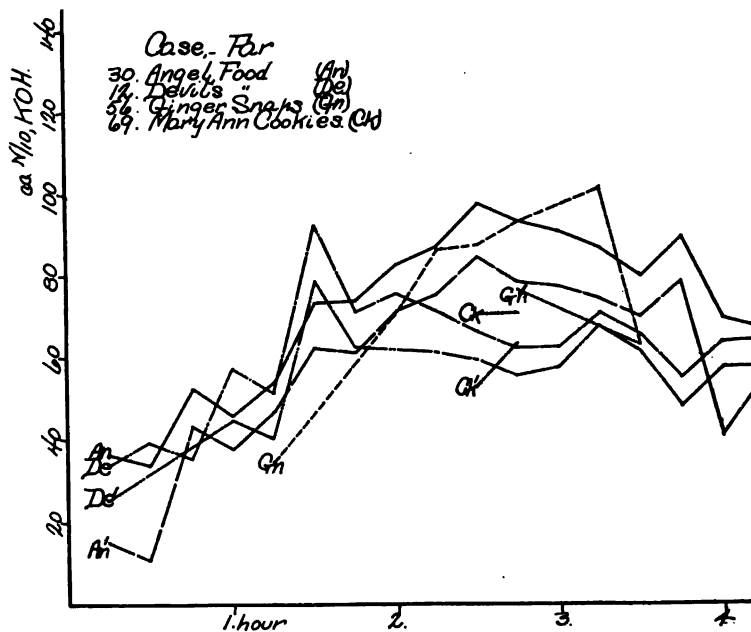


FIG. 12

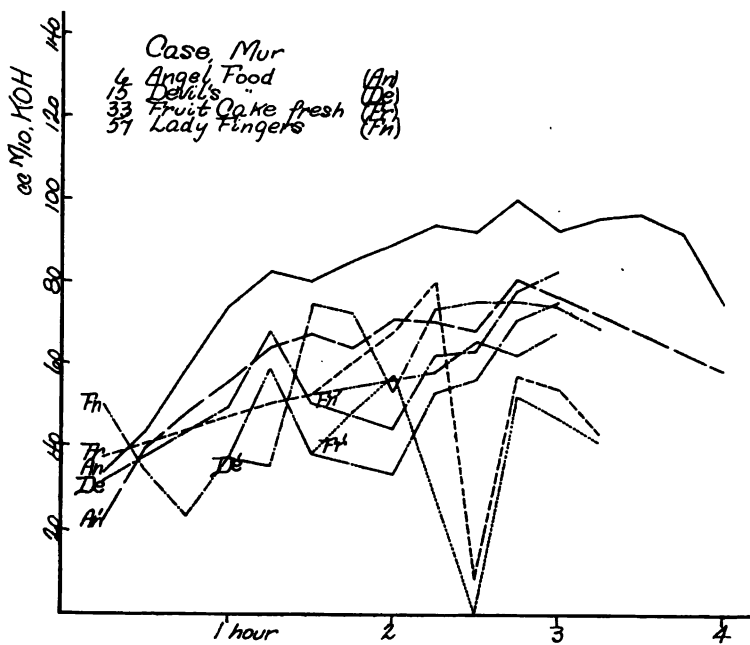


FIG. 13

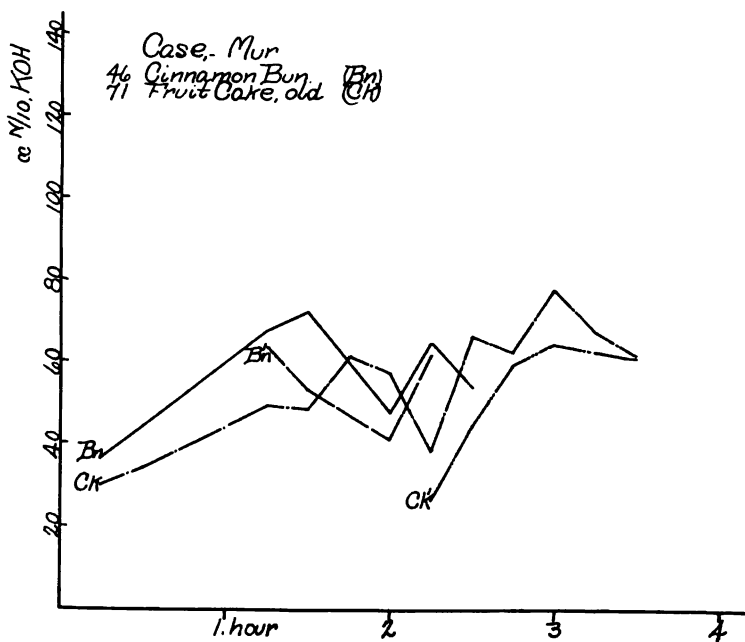


FIG. 14



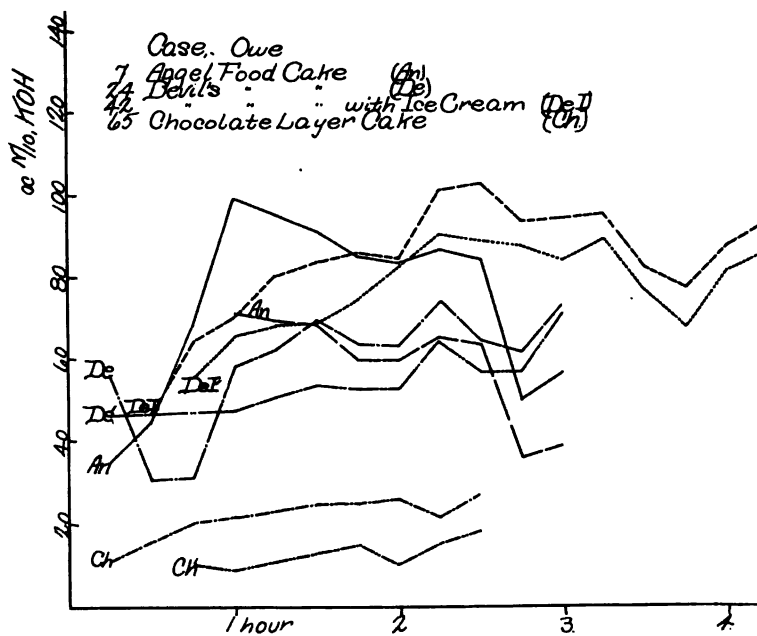


FIG. 15

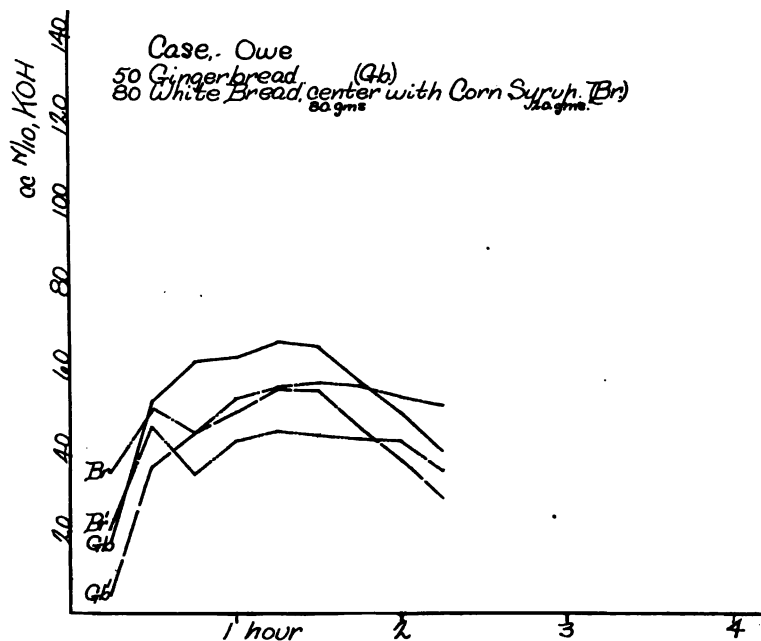


FIG. 16

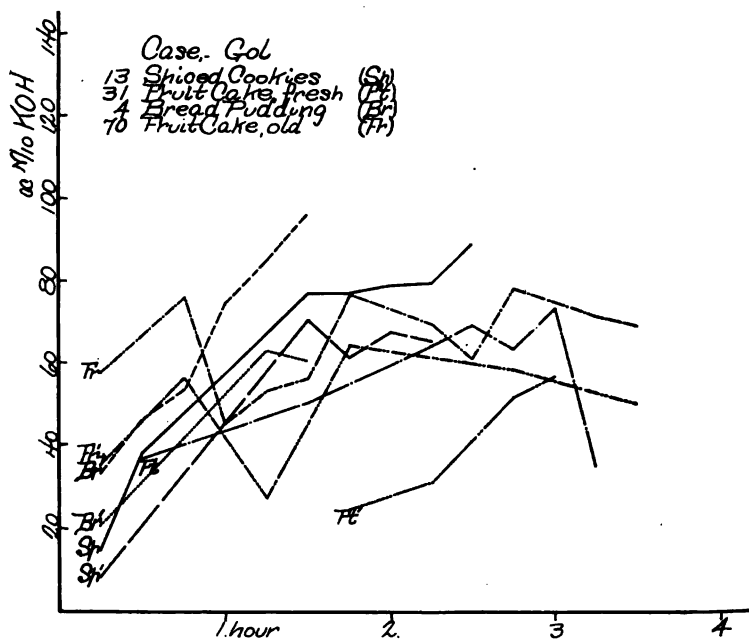


FIG. 17

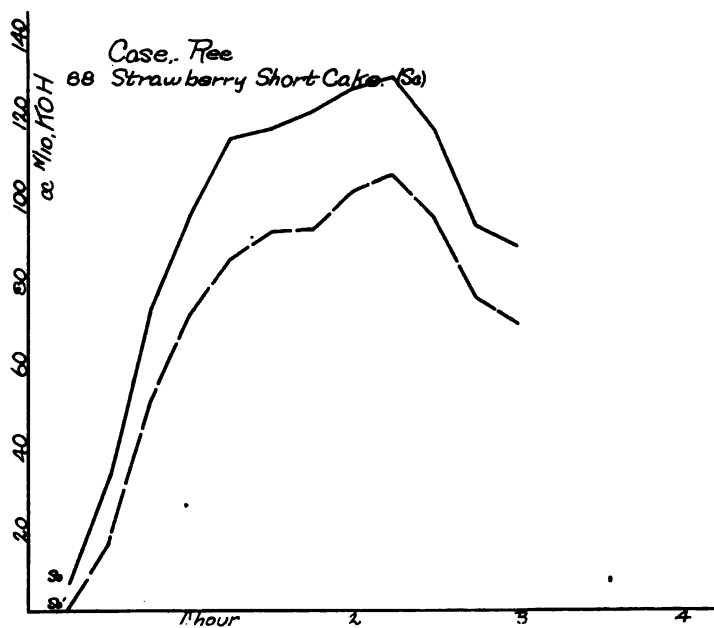


FIG. 18

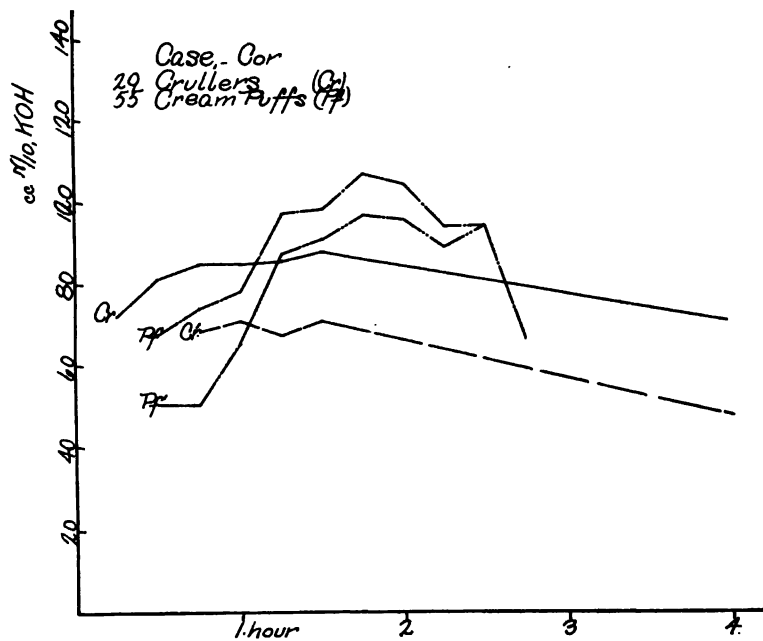


FIG. 19

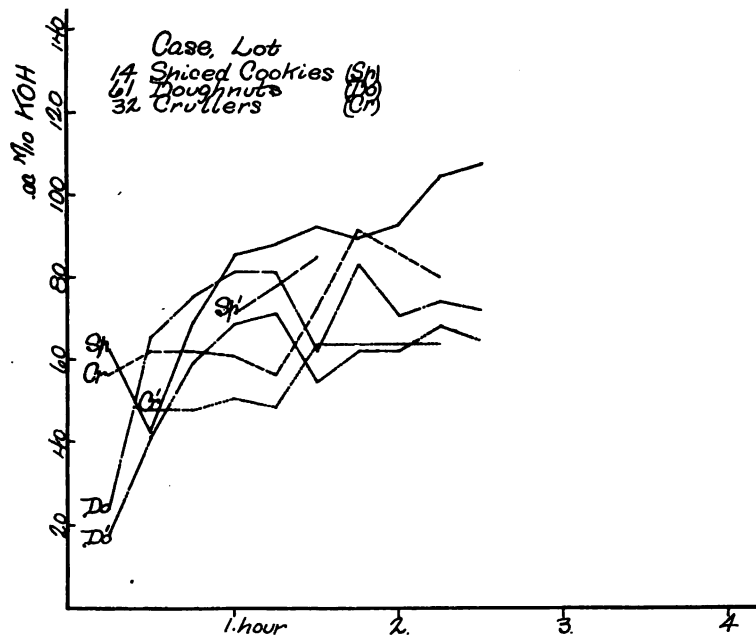


FIG. 20

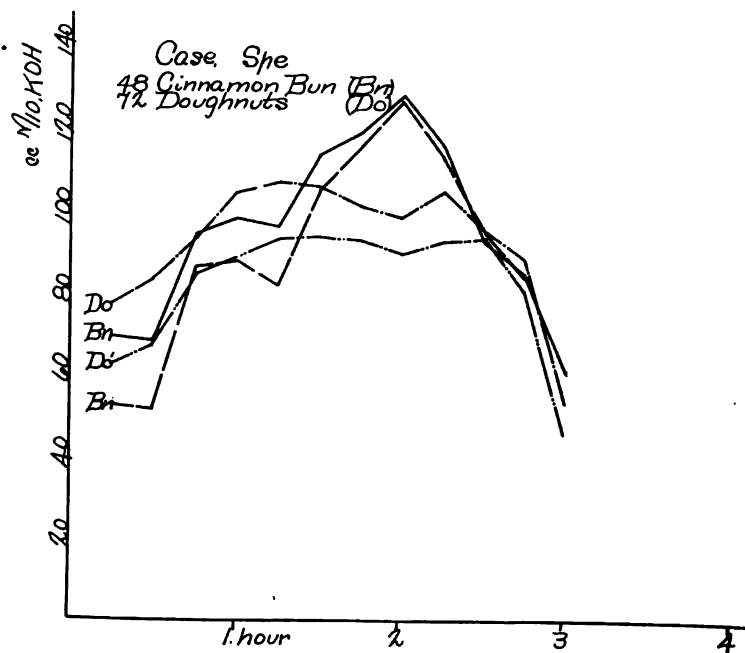


FIG. 21

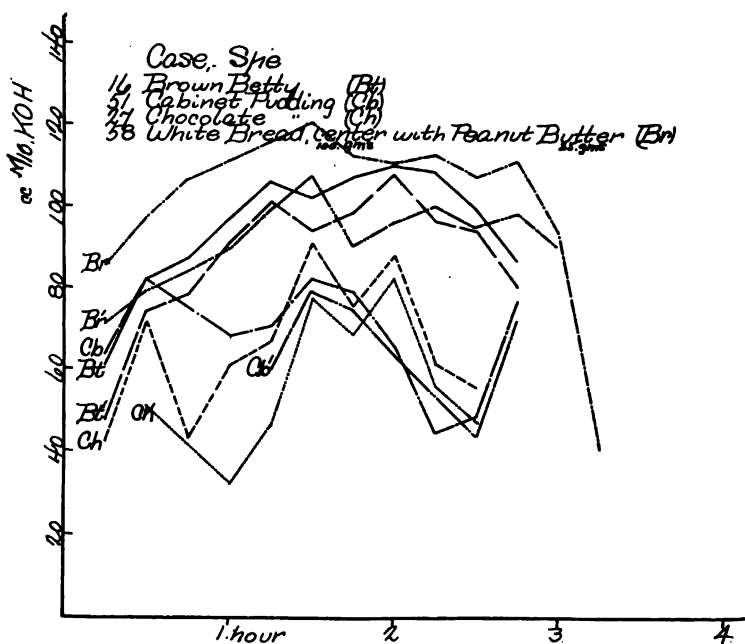


FIG. 22

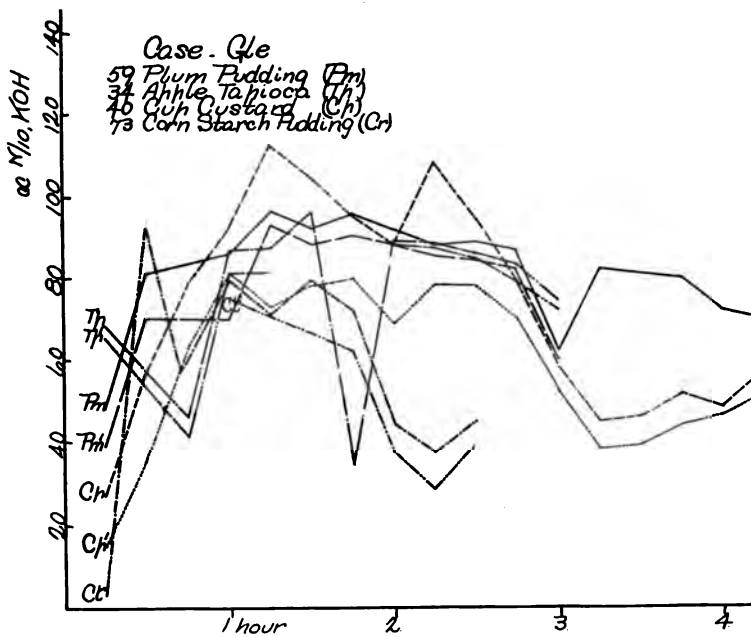


FIG. 23

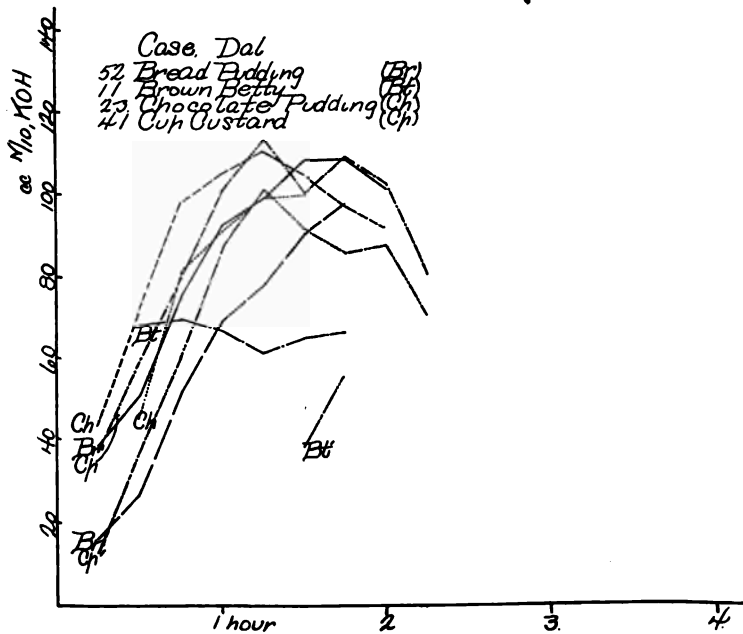


FIG. 24

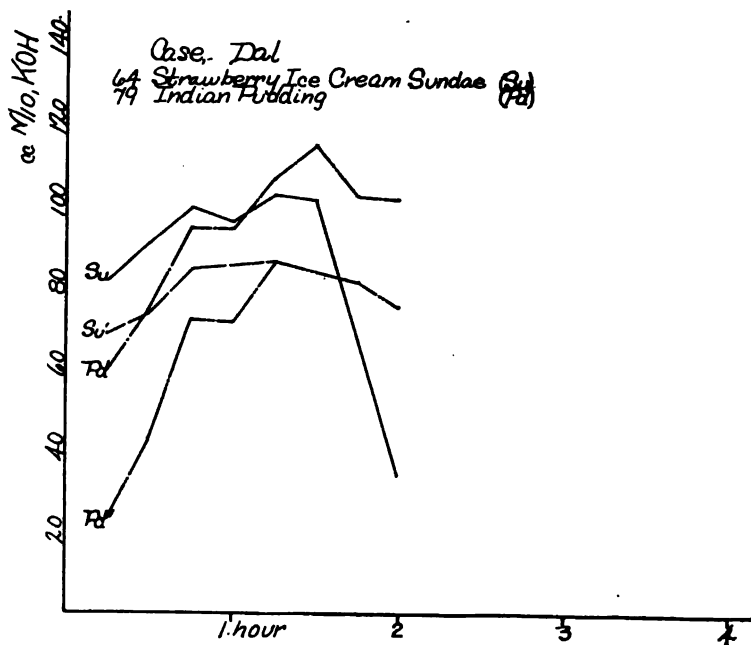


FIG. 25

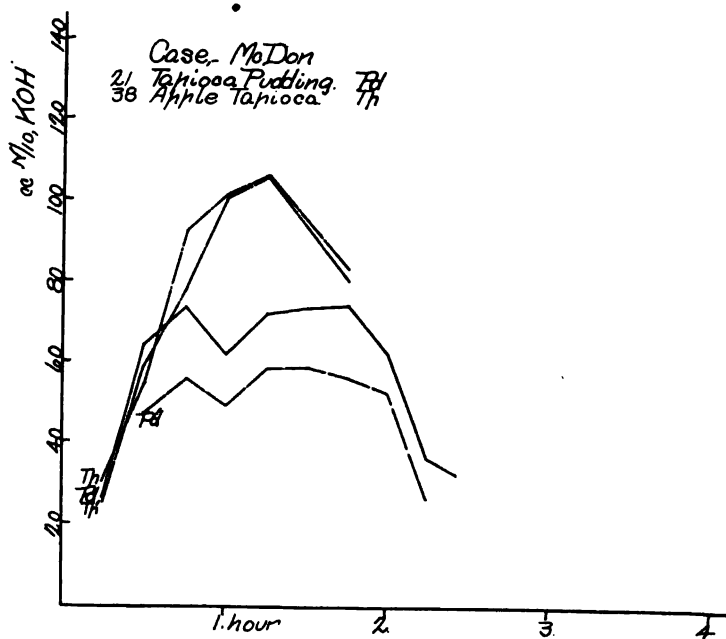


FIG. 26

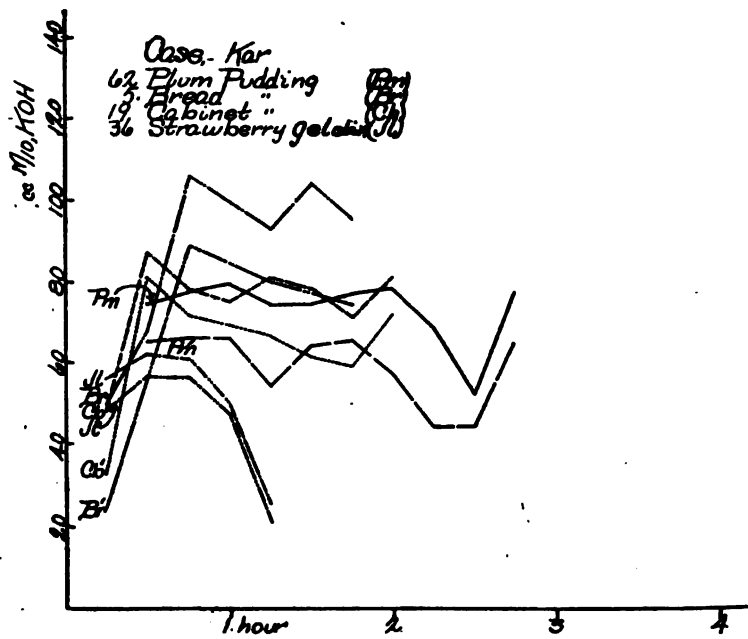


FIG. 27

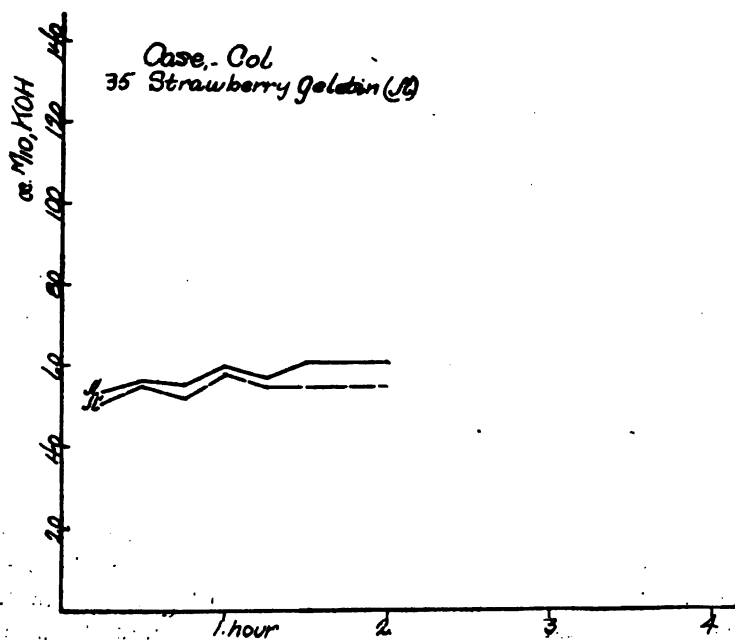


FIG. 28

## SEGMENTAL ACTIVITY IN THE HEART OF THE LIMULUS

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In the structural make-up of the *Limulus* heart the arrangement of the muscular elements into a long tube with well-defined segments, and the grouping of the nervous elements into a median ganglion and lateral nerves, are features which have a significant bearing upon its functional activity. Attention is especially directed to these features, since it appears from the work of Carlson (1) and others that activity in this heart is initiated and conditioned by this intrinsic nervous system, and that contraction in all segments is practically simultaneous. These considerations have suggested the present study, which is an analysis of the contractile process in the different parts of the muscular tube, and of the sequence of activity in the different segments in an attempt to discover the exact temporal relations of the different parts.

In order to make a detailed study of the contraction event it is necessary to employ instruments of unquestionable efficiency. The shortcomings of the suspension method of recording heart action are too well known to need repeating; it suffices to note that records made from the heart of this form by arranging in various ways, so that a thread hooked into one of the lateral arteries may be attached to a cumbersome recording lever, gives a very inadequate representation of the contraction. Small myocardiographs recording by means of Frank capsules were used in the experiments here described, and the arrangement of the apparatus was essentially the same as described by Wiggers (2) and used for analyzing the contraction process in mammalian auricle. The method of adapting it to the *Limulus* heart was as follows: The myocardiograph arms were adjusted to a given segment so as to exactly span the width of the heart at the end of diastole. A fine silk thread was then passed through the superficial layer of the heart at the lateral borders and the arms of the myocardiograph tied thereto. In this manner there was slight traction on the heart until it began to contract.



Moreover, there was very little resistance offered to the shortening as the myocardiograph and also the Frank capsule were covered with very thin rubber membrane.

The record obtained is that of the mid-segmental shortening, the part of the heart that normally exhibits greater contractility than the inter-segmental portions where the ostea are located and in the anterior part the lateral arteries are attached.

*The myogram of different segments.* In considering the relation of the heart of the *Limulus* to its circulatory system it will be noted that the five anterior segments are each connected with outgoing pathways for the blood. If one reasons from analogy to the mammalian heart, a greater contractility should mark the myogram of these parts as compared with that of the posterior segments.

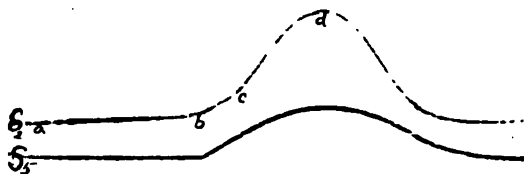


Fig. 1. Simultaneous myograms from heart segments 2,  $S_1$ , and 5,  $S_1$ , showing differences in the contour of the curve from these parts.

A comparison of the myogram from the two ends of the heart, for example, segments 2 and 8, shows differences in the contour, but the curve from segment 5, which has lateral openings, does not exhibit significant difference from that of segment 6, which has no such openings. It appears, therefore, that specialization in contractility, such as is shown in the ventricle of the mammalian heart, is not sharply delimited in the heart of the *Limulus* to that part from which the circulating fluid is outgoing.

The contraction amplitude is nearly always greatest in segment 2. The myogram of this segment, in figure 1, shows a small primary rise,  $b$  to  $c$ , upon which is superposed the main contraction,  $c$  to  $d$ . The upstroke of the principal rise is rapid and the summit is relatively

pointed, indicating that the state of maximal contraction occurs in all of the elements of this segment at practically the same instant. All of these features are exhibited in the myogram of segment 3, excepting that the degree of contraction is usually a little less. The myograms of 4 to 8 all show a smooth rounded contour and, barring differences in the amplitude, the curves from these different segments are practically superimposable. The shortening process in these segments develops slowly, as is shown fairly typically in the myogram of segment 5, figure 1, and moreover the maximal shortening is reached gradually, giving the curve a flattened summit. These features indicate that the contraction takes place in a wave-like manner; some of the elements at the beginning of systole are contracting while others are quiescent, while at the end of systole some are undergoing relaxation while others are still contracting. This form of contraction is suggestive of the type found in the mammalian auricle (2).

In the vigorously acting heart the myograms of segments 2 and 3 are characterized by a slowly developing state of tension preceding the beginning of contraction. This is shown in the myogram of segment 2, figure 1, in the part of the curve from *a* to *b*. The diastolic phase continues so that the curve passes somewhat below the level at the beginning of systole. Then follows the state of developing tonus during which the curve gradually swings back. In a slowly beating heart the curve reaches the level before the next systole begins, but with a more rapid rhythm the tonus increase extends right into the next systole. These presystolic tonus changes have not been found in hearts exposed for an hour or more to experimental procedures; they have also not been exhibited in the records from the posterior segments.

*The sequence of segmental activity.* Inasmuch as the experimental evidence for the Limulus heart favors the view that the impulse is initiated in the median ganglion, the precise time of activity in different segments is a matter of some interest. A casual inspection of a pulsating heart gives the impression of a simultaneous event throughout the nine segments. Carlson (3) states that "when an empty heart has been beating for several hours, or till nearly exhausted and the rate of the pulsations is in consequence much reduced, it can be made out . . . that the contractions start in the posterior third of the heart and travel anteriorly. . . . This is evidently the case in the fresh and vigorous heart." In a later communication the same writer (4), discussing the degree of automatism of different parts of this heart, attributes this function primarily to the middle third, in a less degree to the posterior third, and least in the segments of the anterior third.

From the foregoing statements it is evident that the question of segmental sequence can be answered only by exact measurements of initial activity in different segments. This has been done by simultaneous myograms of selected parts of segments, and by myograms related to the wave of excitation as detected by a string galvanometer. The usual procedure of taking simultaneous myograms was to attach one myocardiograph to segment 2 and a second to segment 3, then after a short interval records were taken. One myocardiograph was then changed to the next segment and the process repeated, and in a similar manner records were taken from all segments. Usually only one series was taken from a heart and sometimes only a few segments were compared, but each record was taken in duplicate and the mean of these was used in making up the data for that experiment.

In general the results show that the middle segments, that is, segments 4 and 5, exhibit activity in a given cycle earlier than the posterior or anterior segments. A comparison of the beginning of contraction of segment 5 and of segment 2 in sixteen experiments shows that segment 5 preceded by an average interval of 0.046 second. In nine experiments comparing segment 2 with segment 4 there is shown an average time interval of 0.047 second, with segment 4 preceding. This indicates that the beginning of contraction in segments 4 and 5 is practically simultaneous and that it normally precedes the contraction of segment 2. On the other hand, a comparison of segment 2 with segment 6, for example, shows in eight experiments an average difference of 0.023 second, indicating that while segment 6 slightly precedes the beginning of contraction of segment 2, it follows by an average interval of 0.024 second the contraction of segment 4.

A comparison of the initial activity in segments 7 and 8 by this method has given less satisfactory results because of the usually low degree of contractility which makes it difficult to determine precisely the beginning of shortening. It is possible, therefore, to make only a general statement concerning them. In six experiments comparing the beginning contraction of segments 2 and 7, it is shown that segment 7 precedes that of segment 2 in four instances and follows it in the remaining two. In a similar way, the data for seven experiments comparing segments 2 and 8 show that the contraction of segment 8 preceded the contraction of segment 2 in three instances and followed it in the remaining four. This indicates that the contraction of the posterior segments normally takes place at practically the same time as the anterior ones.

In order to test the matter of segmental sequence in another way, the excitation process of different segments was taken with a string galvanometer and in turn related to the myogram of a particular seg-

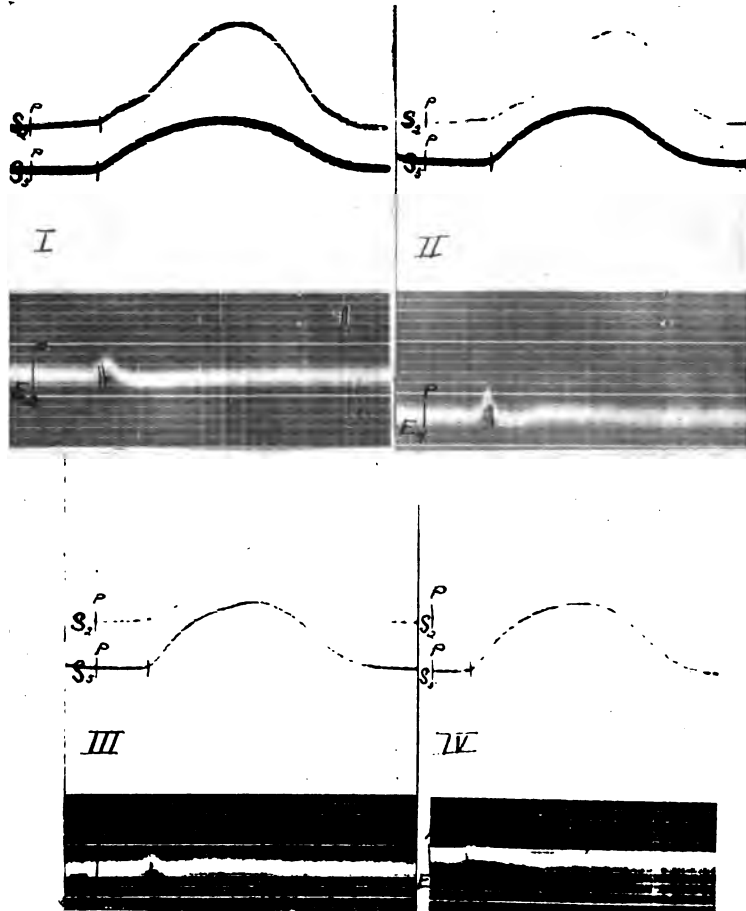


Fig. 2. Electrocardiogram recorded from segments 2, I; 4, II; 6, III, and 8, IV, to show time relation of excitation in these parts to the contraction of segments 2 and 5. Position of points indicated by P. Ordinates in bottom portion of curves designate time intervals of 0.20 second.

ment. The current was led off by placing one electrode on part of the dorsal muscle and the other beneath the heart so that the segment under observation rested upon it.

The characteristic form of the electrocardiogram for this heart has been very well described by Hoffmann (5); little attention, therefore, was given to this feature. In the experiments reported here the beginning of deflection was taken as the index of initial activity, and in figure 2 is shown a series of records which illustrate the time relations for different segments in a fairly typical way by this method. Curve *I* shows that excitation in segment 2 precedes the beginning of shortening in this segment by 0.08 second, and by 0.05 second the initial recorded shortening in segment 5. In curve *II* of this figure it is shown that excitation of segment 4 precedes the contraction of segment 5 by 0.09 second. In a similar manner it is determined from curve *III* that excitation in segment 6 precedes the contraction of segment 5 by 0.06 second, and from curve *IV*, that excitation in segment 8 precedes contraction of segment 5 by only about 0.03 second. Relating these data, it appears, therefore, that the excitation of the median segments takes place about 0.04 second before excitation of the anterior segments and about 0.03 second before excitation of the posterior segments. In this connection it is interesting to note that the distance separating the electrode contact on segment 4 and that on segment 2 was 29 mm., and the time difference in the sequence of activity of these segments, as related to the myogram of segment 5, was 0.04 second. It is evident from these data that the rate of conduction was approximately 72 cm. per second, a figure that is significant in view of the average rate of 40 cm. per second for the conduction rate in the *Limulus* heart nerves, as obtained by Carlson (6) with less sensitive methods.

Mention should be made of the condition met with in some experiments in which the anterior segments showed unmistakably an earlier state of activity than the middle segments. We have in figure 3, for example, a record of contractions of segments 3 and 5 showing the contraction of section 3 preceding by 0.05 second. This proved to be a constant feature of the contraction of this particular heart, since other records in this experiment comparing segments 1 and 6, and 1 and 5, show the same general result. Of the thirty-three experiments in which records have been made this phenomenon has been observed to occur with a degree of constancy in only four hearts. It is significant, however, that records taken from the same heart late in the experiment, after a considerable period of exposure, show initial activity in the median segments.

A careful study of initial activity in the different segments of the *Limulus* heart makes quite evident that the distribution of the excita-

tion process is a rapid event, and that it normally encounters no marked areas of resistance which slow the excitation wave in its spread from the middle region of the heart. Garrey (7) has very clearly demonstrated, however, that this heart is susceptible to the usual types of blocking influences. Moreover, it is a fact well known to all who have worked with this heart that an adequate stimulus applied to any part starts a contraction wave which passes in either direction.

The evidence presented here supports the conclusion, I believe, that normally the contractions of the middle segments precede those of both anterior and posterior segments. The initial tonus changes often

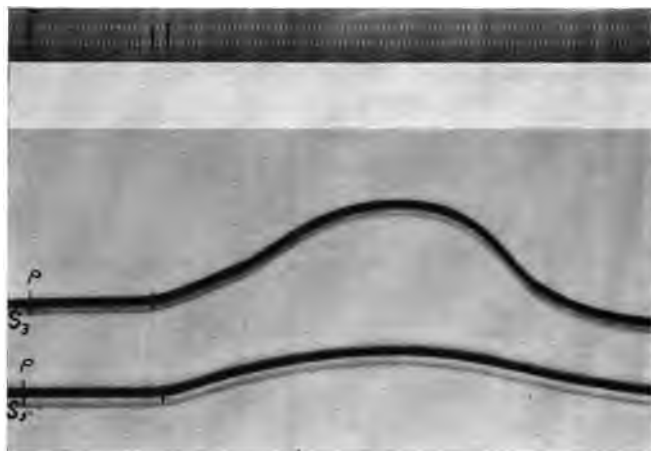


Fig. 3. Myograms from heart segments 3,  $S_3$ , and 5,  $S_1$ , showing an instance in which contraction in the anterior segments preceded that of the middle segments. Position of points indicated by  $P$ . Time curve designates 0.02 second for each full vibration.

exhibited in segments 2 and 3 are significant. It is believed that the increased tonus effects a more quickly acting mechanism in this part of the heart and, coupled with the high state of irritability in a vigorous heart, the recorded activity of this part may precede the initial activity of the relatively less contractile middle segments. The observation of Carlson that a heart, after beating for some time under experimental conditions shows a marked lagging behind of the anterior segments, has been corroborated. But this is taken to mean that the exposure, with the resulting decrease in the nutritive supply from loss of the circulating fluid, favors an earlier appearance of fatigue and blocking in-

fluences in the anterior part because these segments exhibit normally a greater degree of contractility and, therefore, a relatively more rapid using up of the stores of energy.

It has been pointed out in the study of the myogram from different segments that the function of contractility is exhibited in a diminishing degree in the anteroposterior direction; that the heart as it approaches exhaustion shows initial activity in the posterior segments, is interpreted not as the normal sequence of activity but as an expression of the change in irritability which accompanies muscular exhaustion.

#### SUMMARY

1. Myograms, made with small myocardiographs, from different segments of the *Limulus* heart, show greatest contractility in segments 2 and 3 and a diminishing degree posteriorly. The median and posterior segments give evidence of a more gradual development of the shortening of the muscular elements than is shown for the anterior segments, and similarly the end of the contraction process is less marked.

2. The sequence of activity in different segments, by the method of simultaneous myograms and by the method of relating the excitation wave to a standard myogram, indicates initial activity in segments 4 and 5 from 0.04 to 0.05 second before that of segment 2, and in segment 6, about 0.03 second before that of segment 8.

3. In a fresh and vigorous heart there is exhibited a presystolic tonus increase in segments 2 and 3 which appears to hasten the contractile event in these parts so that at times it occurs simultaneously with or even precedes the contraction of the middle portions.

I wish to acknowledge my indebtedness to the Director of the Marine Biological Laboratories for working facilities and to the Director of the New York Aquarium for supplying a part of the material.

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# THE ARTERIAL PRESSURE CURVE AS INFLUENCED BY THE OCCLUSION OF CERTAIN VASCULAR AREAS AND BY HISTAMINE

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It has been shown in a number of researches by Wiggers (1) that the contour of the pressure curve recorded by the membrane manometer affords reliable qualitative data on the dynamics of the circulation. An outstanding feature of this method is that it gives information concerning the failure of normal dynamic relations earlier than the blood pressure results. It has made possible, therefore, a fairly precise designation of the onset of circulatory failure (2).

In the course of studies on blood flow (3) the use of a recording stromuhr made it necessary to close for periods of as much as twenty minutes the main blood vessels supplying visceral organs and in other instances those supplying the extremities. Roy and Brown (4) were probably the first to call attention to the fact that occlusion causes in the frog a dilatation of the arterioles to twice their normal size in two minutes, the effect extending also into the capillaries and veins. Recently attention has been directed to the possible harmful tendency of occlusion measures by the experiments of Mann (5) in which he shows that a shock-like condition can be produced by ligating all of the limb structures excepting the main artery, and Erlanger and Gasser (6) have shown that a similar condition results from a partial obstruction of the inferior cava for about a 2-hour period. In view of these results it is obviously desirable to know whether the less extreme measures used in blood flow experiments produce early changes in the dynamics, also whether there are differences in the character as well as the time of appearance, which distinguish obstructions of central areas from those involving peripheral organs.

There has been included in the program a study of the influence of histamine on the pressure curve, since the observed capillary toxicity of this product suggested a causal relation to the effects following occlusion of vascular areas.



## METHOD

The pressure curve in all experiments was taken by introducing the cannula of the optical manometer into the carotid artery as low down in the neck as possible. A record of mean blood pressure was taken by connecting a mercurial manometer to the opposite carotid artery. This necessitated the closure of two principal arteries but it has been shown that the free anastomosing of the vertebral and spinal arteries permits of compensation through these channels.

Dogs weighing 8 to 10 kilos were used, anesthetized with ether and maintained by closed method with especial care to keep the degree of narcosis very light throughout.

Four different methods of occlusion were made use of: *a*, Ligating of three extremities by placing the ligature about the appendage in such a manner as to exclude the main artery; *b*, clamping of the principal veins to three extremities; *c*, clamping of the inferior mesenteric vein; and *d*, clamping of the inferior cava above the level of the renal veins. In applying the clamp to the inferior mesenteric and the inferior cava a small right abdominal incision was made through which these vessels were exposed and especial care was taken to disturb the viscera as little as possible.

*Ligation of the extremities and clamping of the principal veins to these parts:* In the experiments where ligation effects were studied the extremities were enclosed in the ligatures for a 20-minute period, then for 5 minutes the ligatures were released and in this interval records were usually taken at the beginning and end. The ligatures were then applied again and the procedure repeated. In other experiments clamps were placed on the principal veins and these were manipulated according to the procedure given for the ligatures. The methods differ, therefore, partly in that clamping of the veins does not block certain anastomosing pathways close to the trunk, but mainly in that clamping does not produce tissue injury as does alternate application and release of the ligatures.

The immediate changes when the circulation is reestablished in the extremities are not essentially different in ligating from that when clamps were applied to the principal veins. Usually there is a fall in pressure of not more than 10 mm. mercury with gradual recovery during the 5-minute interval; sometimes there is shown a sharp initial rise and then a fall. The optical tracings taken at the beginning and end of this period show no significant changes.

The records, when viewed as a whole for the seven experiments of the series and the successive stages in each compared, show moderate changes after about 3 hours of the alternate occlusion and deocclusion. All of the experiments were continued for approximately 4 hours. The final contour of the pressure curve usually takes the form of a marked primary oscillation followed immediately by a sharp decline of the curve replacing the systolic plateau of the normal curve. These features are quite characteristically shown in the three segments reproduced in figure 1, in which corresponding points in the curves are indicated by similar letters. These curves were taken as follows: *I* at the outset of the experiment; *II* at the end of 2 hours and 20 minutes during which the alternate 20-minute occlusion and 5-minute release of two femorals

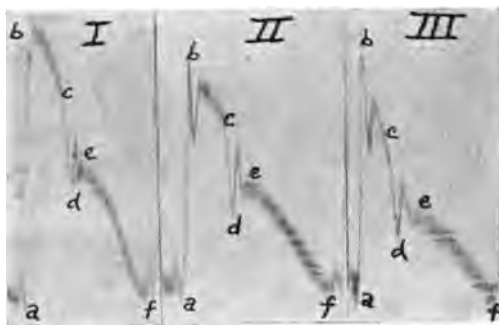


Fig. 1. Segments of the optical arterial curves during the progress of successive occlusion and deocclusion of the veins of three extremities.

and one brachial veins were carried out; *III* at the end of 3 hours and 40 minutes of alternate occlusion and release.

The change in mean blood pressure taking place in this period is slight, but significant in view of the fact that the directional change is downwards and that the optical curve is indicative of lowered peripheral resistance. The following table will assist in bringing out this feature of the blood pressure change and also the time of appearance of a marked change in the form of the pressure curve. This table shows that averaging the interval before a marked change was apparent in the contour of the pressure curve there was required about  $3\frac{1}{2}$  hours of alternate clamping of the veins or ligating of the extremities and that much longer time was necessary to give unmistakable signs of decreased peripheral resistance. Definite indications of circulatory failure and

a shock-like condition were shown, however, in only one instance, that of experiment 8, but the data for this experiment give ground for the view that the occlusion effects per se played a relatively less part than trauma of the sensory nerves from application of the ligatures. There were marked sensory signs with each application of the ligatures and mean blood pressure in the early part of the experiment rose from 172 to 206 mm. Hg.; then occurred a fairly sudden break and a fall in pressure. The optical curve does not give indication of increased resistance as the main cause of this rise; it is probably due, therefore, to an increased cardiac output.

Thus, however viewed, the alternate occlusion and deocclusion of the circulation of three extremities fails to effect changes in the dynamics of a more pronounced character than have been shown to occur in the

TABLE 1

EXPERIMENT	BLOOD PRESSURE		LENGTH OF EXPERIMENTAL PERIOD		ONSET OF CHANGE IN PRESSURE CURVE		STATE OF ARTERIAL RESISTANCE AT FINISH
	At the start	At the finish	hours	minutes	hours	minutes	
1	106	110	5	50	3	45	Decreased
2	74*	118	3	45	3	15	Decreased
8	172	88	4	30	3	25	Very low
10	132	114	3		2	10	Decreased
11	152	132	3	50	3	20	Decreased
14	166	112	5		3	45	Decreased
15	126	112	4				No change

initial stages of circulatory failure of shock (2) and a period well over 3 hours is required for this change to appear.

*Clamping of the inferior cava:* The moderate degree of change in the pressure curve resulting from occlusion of the circulation of the extremities suggested extending the procedure to include larger vascular areas. It is interesting to notice in this connection that the minute volume flow to the posterior extremities, as calculated from the flow in the femoral vein (7), is 45 cc. There are no data available concerning the supply to the anterior extremity but it is usually assumed to be approximately equal to that of the posterior extremity. The three extremities, therefore, would represent a volume flow of 135 cc. per minute. On the other hand, it has been shown that the kidneys have a minute volume flow of 195 cc. for the two organs (8). These data represent average normal values and at best are only approximate,

but they serve to indicate that the clamping of the cava affects a many times greater vascular field than was included under the experiments on three extremities.

The pressure changes following cava obstruction indicate in a striking way that there is a greatly diminished venous return which causes some cardiac embarrassment. There is a reduction in the amplitude of the curves with a retardation in the gradient of the upstroke and the end of systole is not sharply marked by a distinct incisura. With the first deocclusion of the cava there is evident in the pressure curve a resumption of the main characteristics of the original, but after the second period of clamping little tendency to recovery is shown; furthermore, there is indicated a marked relaxation in peripheral tone. The total time of occlusion of the inferior cava necessary to give these indications of a break in the dynamics was never more than 1 hour. It may have been somewhat less since the periods of occlusion were from 25 to 30 minutes.

The results of one experiment in which the clamp was applied just posterior to the inlet of the renal veins is of interest, since in this manner the kidney circuit is left intact. The blood pressure fell about 30 mm. Hg. when the clamp was on but showed practically complete recovery each time it was removed. This was repeated four times, covering a period of 2 hours and 10 minutes, and at the end of this time the optical curve showed the characteristics of initial failure. There is suggested, therefore, a degree of proportionality between the time of appearance of these circulatory changes which influence the form of the pressure curve and the extent of the vascular field occluded.

*Occlusion of the inferior mesenteric vein:* The inferior mesenteric vein drains an entirely visceral area in contrast to the conditions considered above. Moreover, it represents a vascular field as indicated by the figure for blood flow of 164 cc. per minute (9) quite comparable to that studied under occlusion of the extremities.

The outstanding features in the pressure curve during the progress of this type of occlusion are the relatively sudden appearance of the change in the contour of the curve and the slight indication of progressive development after this initial change. The first period of clamping was about 40 minutes and the pressure curve shows (fig. 2, II) at the end of this time a sharp primary oscillation (*a-b*) without plateau (*b-c*) and with low diastolic limb (*e-f*). Each record was taken at the end of the 5-minute period of deocclusion. Segments III to VI, while differing somewhat in amplitude, show slight changes in their essential

characteristics and give evidence of a comparatively fixed state of dilatation after the first period of stasis. Furthermore, these succeeding periods of occlusion show only moderate depression in blood pressure and nearly identical recoveries during the periods of deocclusion. These changes lend support to the view that the cardiac mechanism is not markedly affected by the degree of reduction in the venous return.

The significant features in these results appear, therefore, as the rapid initial decrease in blood pressure with comparatively small changes from the alternate periods of occlusion and deocclusion, and the slight tendency to a progressive type of change in the pressure curve. Many factors are concerned probably in the production of the effects following occlusion of the venous return from such vascular areas, but the

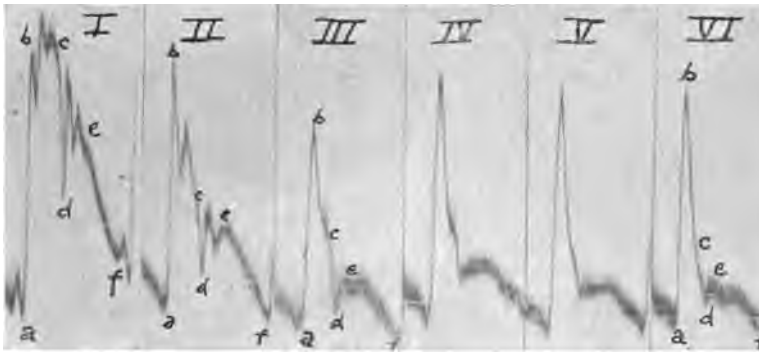


Fig. 2. Segments of optical arterial curves during the progress of successive occlusion and deocclusion of the inferior mesenteric vein.

following warrant special consideration: (a) Mechanical distention of the blocked venous system; (b) a toxic action of products formed in the stagnant areas which exert further injurious effects when periodically flushed into the circulation; and (c) the damaging of the vascular walls with accompanying alterations in permeability. The present experiments do not permit of direct conclusions concerning which of these factors plays the essential rôle in the production of the effects studied. Indirectly they give evidence, however, in favor of the view of mechanical dilatation as the main cause of these primary changes, since it has been shown that occlusion of an intestinal area which affords by virtue of its plastic character a favorable site for mechanical stretching of its vascular walls, is immediately affected and the change appears almost in full magnitude at the outset. The occlusion of a peripheral

field of comparable vascular magnitude, which presents a comparatively rigid vascular area, gives results essentially different in that corresponding changes in the pressure curve appear much later in the course of events.

Thus far, no account has been taken of the possible toxic effects of substances formed during the periods of stagnation. In order to throw light directly upon the probable degree of influence which substances of this character exert the experiments below with histamine were performed.

*Successive injections of histamine:* The results of Dale and Laidlaw (10) show quite conclusively that the administration of histamine may produce a condition simulating traumatic shock. This phenomenon

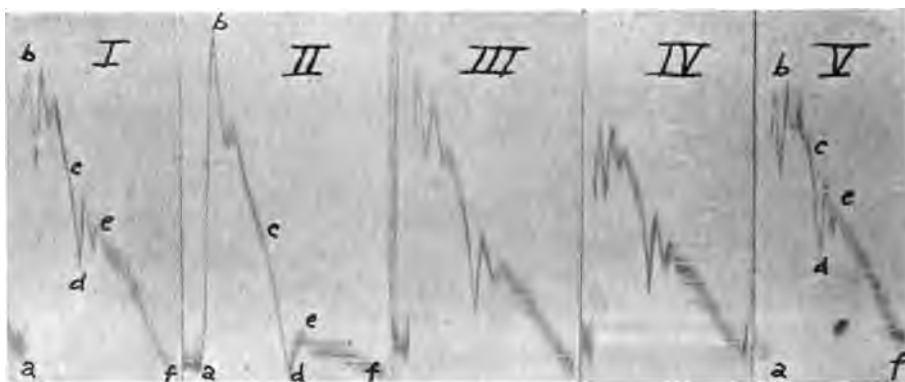


Fig. 3. Segments of optical arterial curves at certain stages during the blood pressure change from injection of 0.15 mgm. histamine. The numbering of segments corresponds to positions indicated on figure 4.

they assign to increased capaciousness through the relaxation of capillary tone from the toxic action of this substance. In their experiments cats were used for the most part and a dosage of a milligram per kilo body weight was found necessary to produce circulatory collapse.

In the present experiments on dogs it was sought to test not only the immediate effects but also the accumulative action of histamine on the dynamics of the circulation; accordingly, the injections were usually 0.04 or 0.05 mgm. (the ergamine of Burroughs, Wellcome & Co.) repeated at 10-minute intervals over a period of 3 to 4 hours. Such a dosage never fails to give at each injection a fall of over 50 per cent in blood pressure at the start. The recovery is rapid at first and usually complete in less than 3 minutes but as the experiment progresses and

also following larger injections a somewhat longer time is required before the pressure returns.

In figure 3 are shown portions of the optical records taken during the changes produced by the injection of 0.15 mgm. histamine. These curves, taken in connection with the tracing of the blood pressure change shown in figure 4, portray the immediate effects of this substance upon the pressure relations. Coincident with the fall in mean blood pressure the optical curve shows (fig. 3, segment *II*) a large primary oscillation

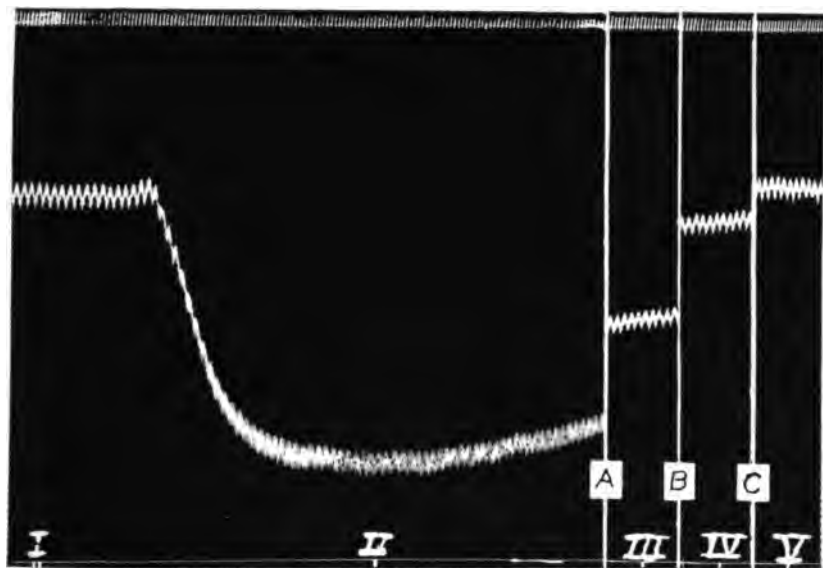


Fig. 4. Tracing showing blood pressure change as related to optical curves in figure 3. Numerals correspond to segments in that figure. The intervals A, B and C correspond to 2 minutes, 5 minutes and 5 minutes, respectively. Time curve indicates seconds.

(*a-b*), indicative of a collapsed state of the arterial system (*c-d*), and a flattened diastolic limb (*e-f*), signifying a low state of peripheral resistance. There is a rapid restoration of peripheral tone indicated in segment *III*, *e-f*, by the immediate rise in this portion of the curve. This change in resistance suffices to restore mean blood pressure in a short time but it will be seen from segment *IV* that the recovery of the dynamic relations is complete before mean blood pressure has reached its original level. The extent to which cardiac output is influenced by

these changes does not appear from the present data. Further experiments dealing with this feature are in progress.

Turning now to the influence of the successive injections, it was demonstrated at the outset that small doses, e.g., 0.01 mgm. per injection, have little accumulative effect. With individual injections in amounts of 0.05 mgm. it has been possible generally to produce in dogs of about 8 kilos signs of initial circulatory failure after a total of 0.5 to 0.7 mgm. had been injected in the manner indicated. The stages in the failing dynamics are very well brought out in figure 5. Segment *I* was taken at the outset of the experiment and serves as a basis for comparison; segment *II* was taken after seven 0.04 mgm. injections had been given; and segment *III*, after thirteen such injections. Close

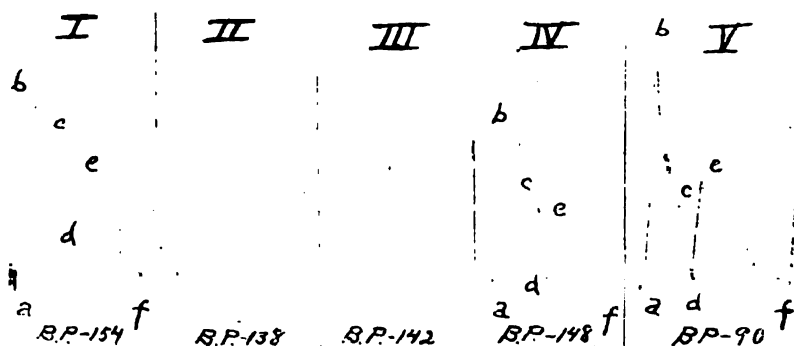


Fig. 5. Segments *I*, *II*, *III* and *IV*, optical arterial curve during progress of successive injections of histamine. Segment *V*, taken 25 minutes following injection of 5 cc. adrenalin (1:5000). Description in text.

inspection of these curves shows a sharper contour in *II* and *III* and a slight diminution in the height of the primary oscillation. These features are significant in that they anticipate the more marked changes characteristic of failing peripheral resistance and that they take place without significant changes in mean blood pressure. At this stage of the experiment the dosage was increased. There followed two 0.2 mgm. injections at the usual 10-minute intervals and one injection of 0.5 mgm. Whereas the smaller amounts had usually caused a fall in blood pressure to 50 to 60 mm. Hg., the latter dose depressed the pressure to 36 mm. Hg. Segment *IV* was taken 20 minutes following the 0.5 mgm. injection and at a time when blood pressure had practically recovered. It will be seen that the introduction of the larger amounts augmented the features already noted and that the curve now exhibits



a certain likeness to that shown under the occlusion of the veins to the extremities and characteristic of initial failure.

These results present quite typically the effects on the optical curve of repeated injections of histamine. It is significant to note, also, that all of the experiments were continued for a period of about 4 hours without a single instance of circulatory collapse appearing, notwithstanding injections at the conclusion of some of the experiments of a milligram of histamine following closely upon successive doses only slightly less, with a total given during the experiment amounting to 3 mgm. or more. These observations lend support to the view that the dilatation resulting from the amounts of histamine used, and administered in the manner indicated, causes only moderate dynamic changes. Furthermore, it appears that the change is one that may be due to the reduced resistance from simple capillary dilatation. With the possibility in mind that a moderate vasoconstriction might counteract the effects of the periodic histamine dilatations and thereby restore the failing dynamics, injections of 5 cc. of adrenalin (1:5000) were given at the rate of about 1 cc. per minute. The usual constrictor response followed but passed off in ten to fifteen minutes and a vascular equilibrium was again established. The pressure relations at this stage are very well shown in segment V of figure 5, taken 25 minutes following the administration of the adrenalin. It shows that, notwithstanding a good state of peripheral resistance signified by the height and slope of the diastolic limb, *e-f* of the curve, there is a diminished distention of the arterial system evidenced by the increased amplitude and sharp contour of the primary oscillation, *a-b*.

The marked vasoconstriction of the adrenalin appears to have the effect, therefore, of augmenting the condition started by the histamine. In this connection it is interesting to consider briefly the possible mechanism of action of adrenalin whereby it accentuates the dilatation initiated by histamine. Hartman and McPhedran (11) state that large doses of adrenalin dilate the intestinal vessels. On the other hand Erlanger and Gasser (12), while not able to agree with this conclusion, point out that portal pressure is greatly increased by giving large amounts.

The results presented above give unmistakable evidence of a decrease in peripheral resistance after repeated small doses of histamine. It is well to bear in mind that several factors may contribute to this end, important of which are decreased arterial tone, dilatation of the capillary areas and decreased viscosity of the blood. While it is not possible

to state with certainty regarding which of these factors plays the greater rôle in the reduction of peripheral resistance with histamine, it is reasonable to assume that the action of adrenalin described above is due to a further dilatation of the weakest link in the vascular circuit. The usual absence of reduction in arterial tone from adrenalin, and also the excellent results of Dale and Richards (13), indicating that histamine is a capillary dilatant; support the conclusion that the capillary system is the part less resistant and is the area which compensates for the intense constriction of the adrenalin. The data at hand do not indicate whether this effect is mainly on the intestinal area or is generally present in the capillary blood-bed of all organs. The observation of a great increase in portal pressure lends support to the view that the area supplied by this vessel may play the larger part in these results.

One point still remains to be discussed; that is, the bearing of the results with histamine upon the effects following occlusion of different vascular areas. The optical curves show features in common, each presenting changes indicative of initial failure of normal dynamic relations. It might appear, therefore, that the occlusion effects were caused by metabolic products with histamine—like action formed during the periods of stasis. There seems little doubt that such products may contribute to the effects of occlusion, but that they are not the main cause is evidenced, I believe, by the relatively large amounts of histamine necessary to initiate failure changes and by the observation recorded above that during the periods of reëstablished circulation through the occluded part there are never indications of a dilating substance being carried into the general circulation.

#### SUMMARY

1. Alternate periods of occlusion and release of the circulation of three extremities, when continued for about 4 hours, produce changes in the optical pressure curve comparable to those shown in initial circulatory failure. There appears to be little difference whether the occlusion is produced by *a*, ligating all of the limb structures except the main arterial supply; or *b*, clamping the principal venous channels from these organs.

2. Clamping the inferior cava anterior to the renal veins shows indications in the pressure curve of cardiac embarrassment from diminished venous return. Two 30-minute periods of occlusion of this vein interrupted by 5-minute intervals of reëstablished flow produce

marked changes in the dynamic relations. By lessening the vascular area blocked, the time of appearance of these changes is somewhat delayed.

3. Blocking the venous return from a visceral area brings on a failure type of pressure curve much sooner than occlusion of a peripheral area of comparable vascularity.

4. The observations of a slow, progressive development of the occlusion effects of peripheral structures, the rapid onset with little evidence of progressive development for visceral areas, and the character of the changes portrayed in the pressure records, support the interpretation that the cause is essentially one of mechanical distention of the blood-bed in the occluded parts.

5. The optical pressure curves during the blood pressure change from a small dose of histamine show a sudden decrease in the peripheral resistance with a greatly diminished arterial distention, and an immediate recovery of peripheral tone. Injections of 0.04 to 0.05 mgm. repeated at 10-minute intervals produce after many such doses a small degree of decrease in peripheral resistance. Larger doses serve to accentuate these initial effects of the periodic dilatations.

6. Adrenalin augments the failure changes initiated by the repeated histamine injections.

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# THE ARTIFICIAL PRODUCTION OF MONSTERS DEMONSTRATING LOCALIZED DEFECTS AS THE RESULT OF INJURY FROM X-RAYS

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In a recent paper (1919)<sup>1</sup> the author has detailed the results of a series of experiments upon frogs' ova where, by the use of X-rays generally applied to the surface of the ovum, a uniform defect was produced in the embryos. Inasmuch as a fixed quantity of energy was concentrated upon the ovum in the early stages of its development, practically all of the embryos presented the same gross and microscopic structural defects. In this present series of experiments, however, the X-ray energy was permitted to act upon a relatively small amount of the egg substance through the utilization of a perforated lead screen interposed between the tube and the specimen. The energy was derived from a large Coolidge tube which carried a milliamperage of 50 at 50 kilovolts. The interposed lead diaphragm measured about 24 mm. in thickness, through which a hole 0.3 mm. in diameter had been bored. Fertilized eggs of the frog, in a developmental stage no later than the two-celled, were placed behind the hole in the diaphragm in line with the center of the target of the tube and at a distance of 22.5 cm. from it. By this arrangement a maximum effect of X-ray energy upon a relatively small amount of the protoplasmic mass of the egg was brought about. An effort was made to so place the eggs that the energy entered at a point in the animal hemisphere and emerged at a corresponding point in the vegetable hemisphere. A columnar mass of protoplasm, 0.3 mm. in diameter, extending from the upper to the lower surface of the 1.7 mm. ovum, was subjected thereby to a maximum amount of direct radiant energy. In each instance an exposure varying from two to four minutes was

<sup>1</sup> The artificial production of monsters conforming to a definite type by means of X-Rays. *Anat. Rec.*, xvii, no. 3, November, 1919. A bibliography list is appended.

made. The eggs were then transferred to a specimen jar containing 1000 cc. of tap water and permitted to develop at room temperature, the water being changed frequently.

It was ascertained after repeated experimentation first, that the amount of X-ray energy passing through the thickness of the 24 mm. screen at the distance given, and under the conditions of exposure mentioned, exercised no injurious effect whatever upon normal ova. Likewise, it was demonstrated that the secondary radiation arising from the edges of the hole in the diaphragm was so small in amount as to be a negligible factor. The effects recorded in these experiments were owing apparently, therefore, to the direct action of the X-ray energy upon the protoplasm of the egg using this term in its broadest sense, in the restricted region mentioned.

A study of the external features of the developed embryos reveals but little in comparison with the microscopic findings. In some instances it may be detected that the development of the eye or of the ear or both upon the same side is defective. Occasionally, as well, the gill tuft upon one side is absent. Furthermore, a lateral curvature of the body at the junction of the head with the trunk or at the level of the trunk, or at the level of the junction of the trunk with the tail may be observed, but this curvature is no greater than that which occurs with many normal tadpoles which are fixed together in large numbers. Some show an asymmetrical position of the dorsal fin at the level of several adjacent segments of the trunk. On the other hand, the color of the epidermis is normal throughout. The length, breadth and thickness of the tadpoles have not suffered to any appreciable degree over the controls.

A study of the serial sections of the tadpoles demonstrates, however, a peculiarly abnormal condition of the anlagen of several segments of the trunk and neck regions. As the sections are traced in sequence from the head to the tail, at a level beginning ordinarily at the optic chiasm and extending caudally to the region of the stomach, a marked difference is to be observed when the corresponding portions of the embryo to the right and to the left of the notochord are compared. Upon first glance the impression gained is that of unusual asymmetry of the embryo in which the neural tube, the notochord and the enteron have left their customary median position and migrated together toward one or the other side. The space ordinarily occupied by the mesodermal tissue and its derivatives is considerably smaller in area upon the side toward which the notochord has migrated than upon the

opposite side. At such levels the neural tube and the notochord, while occupying the real median plane of the embryo so far as anlagen are concerned, actually lie nearer one lateral trunk surface than the other by reason chiefly of the above mentioned inequality of development of the mesodermal derivatives. This inequality of mass distribution is the result of a retardation of development of certain consecutive hemisegments of the embryos. In no instance is this phenomenon restricted to one segment. Most often it involves those located immediately caudal to the point of origin of the optic stalk from the brain, and usually the defect extends as far caudally as the stomach.

A close microscopic examination of the cells in this defective area demonstrates the point that the various organ anlagen are not absent, but the processes of differentiation in the cells constituting them have been so greatly checked by the X-ray energy in contrast with those of the corresponding anlagen of the opposite half, that they present an appearance of the greatest degree of developmental retardation. The individual cells present certain features of both cytoplasm and of nucleus, moreover, which are unmistakable indications of deviations from the normal morphological characters of these cells.

The abrupt shifting of the neural tube, of the notochord and of the enteron from their normal median position in the mass of the embryo gives rise to the appearance of a lateral flexure of the trunk at the level of injury. In the caudal portion of the affected area a similarly abrupt reverse flexure brings these structures back to their normal median position. A singular feature of the abnormal embryos consists in the absence of any indication whatever of inflammatory reaction to injury in the affected area. Similarly the absence of extruded cells and of protoplasmic degenerations, involving either nucleus or cell body, may be interpreted as indicating a type of injury not of the most severe grade, as that noted by the author in his previous paper.

The cytoplasm of the cells in the affected area possesses a large, more-coarsely granular appearance than is normal. Pigment granules which, in the later stages of development, are more completely restricted to the epidermal cells, are to be observed in those cells the substance of which lay in the path of the rays through the ovum. Their presence in numbers enables one to mark out readily the direction of this path in the sectioned embryo. The nucleus of these cells presents, in the main, two general departures from normal; first, the chromatin is clumped either into large, deeply-staining masses restricted for the

most part to the periphery of the nucleus, leaving the center in contrast comparatively clear; or, secondly, the chromatin is more finely granular, more uniformly distributed, but invariably much less intensely staining than normal. It might be inferred that these two features represent different degrees of the same type of injury. There is no difficulty encountered, however, in readily differentiating between normal and rayed cells through these features. Furthermore, the contrasted appearance of these cells to normal cells in these partly-rayed specimens is all the more readily appreciated since both types may be seen in the same microscopic field.

In the previous paper on type embryos, the presence of nuclear and of protoplasmic detritus in the body cavities, such as the enteron, neurocele, optic vesicle, etc., was interpreted, as had been done by Hertwig previously with radium embryos, as a certain indication of such a degree of severe injury as to eliminate these cells from participation in the subsequent developmental stages of the embryo. Such features in this present series are, however, as was mentioned above, entirely absent. The injury produced by the rays was not sufficiently intense, apparently, to destroy the vitality of any of the cells. There ensued, on the contrary, a temporary suspension of differentiation followed by a lengthened tempo in the developmental rate of these affected cells. The absence of a reactive inflammation is, at present, difficult of interpretation unless we assume that possibly the damage wrought by the rays has been wholly intracellular.

As the different systems of the embryo are taken up in order and studied through the serial sections, several facts assume significant importance dependent upon the degree of reaction to X-ray influence. The ectoderm in the areas not in the path of the X-rays appears normal in thickness and in the disposition of its cells. Where the pencil of rays has passed through the embryo, however, those ectodermal cells in line with it depart from the normal in structure most markedly. In this area the entire thickness of the ectoderm in general is markedly increased. The individual cells are larger and the nuclei more deeply staining. The cell borders and the nuclear outlines are, however, distinct. This increase in thickness is, however, not uniform throughout the area affected, there being produced as a result an unevenness in the external surface of the ectoderm which amounts to a corrugation or wrinkling of this layer without involving, however, the basement membrane. In many instances the ridges so produced are hollowed out through the presence of channels which run a greater or less dis-

tance in a direction generally parallel to the underlying surface of the ectoderm. This reaction to X-ray influence resembles strongly that referred to by the author in his previous paper and noted as well by Hertwig in his study of radium embryos. There is no evidence of actual death or of desquamation of any of the ectodermal cells, neither are there indications of inflammatory reaction. In no specimen has the thickness of the layer been reduced.

As the brain and spinal cord are followed through the sections, there is to be noted at the level of injury a marked dwarfing of the brain vesicle upon the affected side. What appears to be a compensatory hypertrophy of the vesicle wall upon the unaffected side has resulted in a shifting of the median ventral furrow in the floor of the neurocele toward the affected side. This accentuates the appearance of asymmetry. The neuroblasts upon the unrayed side are normal in size, in shape and in the appearance of their nuclear and cytoplasmic material. Stratification ordinarily has progressed to such a degree that the external fiber layer may be distinctly differentiated from the nerve-cell layer. Upon the affected half of the vesicle there is a remarkable dwarfing of the neuroblast layer. The size of the individual cells is remarkably reduced, the nucleus is small and but faintly staining, and the protoplasm is but slightly granular. The sectional cross-area of the cell-mass constituting the floor of the vesicle is less than one-half that of the unaffected side. The dorsal median ridge in the roof of the brain occupies, however, its normal position. But very few bits of extruded nuclear or protoplasmic material are to be found in the neurocele. As the tube is traced cephalically or caudally, it is found to make a sharp, almost right-angled flexure away from the affected hemisegmental area, this flexure being so sharp when seen in the cross-sections as to appear as almost a longitudinal section. The purpose of these bends is naturally to bring the tube back into its normal median position in the unaffected segments of the body.

Dependent upon the level of the segments involved, either the optic vesicle or the otic vesicle, or both, upon the same side may be involved in the injury. Where the former has been encountered by the X-rays the cells constituting the optic stalk and the vesicle demonstrate the same abnormal features of nuclear and of protoplasmic structure as those presented by the neuroblasts upon the affected side of the brain. Stratification of the lining cells is absent; the lens is ordinarily not formed. These same features may be detected as well when the otic vesicle is involved in the injury. Where, however, a greater amount



of X-ray energy has acted upon these two organs, they are then entirely absent. The optic vesicle may be reduced to a mere stump attached to the ventral surface of the brain. There is no indication given through the thickening of the ectoderm of the formation of a lens. As was noted in the brain cavity, there is but little indication of the presence of extruded cells or of nuclei in these two vesicles.

The shifting of the notochord from its normal position toward the affected side is brought about by the same abrupt angularity of flexure as was noted in the neural tube. By reason, however, of a greater degree of migration, its normal ventral relationship to the tube is somewhat altered. There is nothing, however, to be noted as abnormal either in the appearance of its cells or in their arrangement.

Since the injury to these embryos is restricted to the neck and cephalic segments, the only portion of the enteron demonstrating abnormalities is the pharynx. In those segments where the injury has been severe but half of the pharyngeal wall is present. No effort has been made by the organism apparently to remedy the defect through the completion of the defective half of the tube-wall. The lining cells upon the affected half remain small, spherular and isolated. The number of them is greatly reduced and an orderly stratified grouping is completely absent. The general arrangement of these cells corresponds to that found in a very early stage of development. Differentiation of these cells, as was true also of the neuroblasts and of the cells of the optic vesicle, has been practically completely suspended. Upon the unaffected side, however, the pharyngeal wall is distinctly clothed by well-differentiated cells corresponding to the period of development. The contrast between these two halves of the pharyngeal wall is one of the most striking features presented by the embryos.

Similarly, the pronephros in the affected area is reduced greatly in the number of its tubules and in their size. The individual cells lining the walls of these tubules present features corresponding to those seen in the affected half of the pharynx, i.e., irregularity in arrangement together with spherulation and isolation of the cellular elements.

Cephalic to and caudal to the affected area the muscle segments are normally symmetrical in point of differentiation and of growth. In the affected segments, however, the myoblasts may be sharply contrasted from the standpoint of differentiation. The pigmentation of these cells, present normally only in early stages of development, is retained and, as well, the spherular, isolated, non-differentiated characters of the cells are distinctive. Upon the unaffected side the myo-

fibrillae may be readily distinguished. The myoblasts, which at this stage are elongated, have assumed their definitive arrangement with respect to the long axis of the embryo. These cells are sharply contrasted developmentally with those of the affected side. The cross-sectional area of the affected half of any myomere approaches ordinarily but less than half that of the unaffected side.

But few or no abnormal features may be discerned either in the heart or the pericardium. The great vessels passing through the rayed segments, on the other hand, demonstrate certain abnormal features. Most prominent among these is the retarded, embryonic condition of the lining endothelial cells and the undifferentiated condition of the tunics of the vessel walls. In only the largest of these have they made their appearance. Moreover the smaller blood vessels are entirely absent. The cellular elements which should assist in the formation of them remain small, spherular and isolated. There are no coördinated attempts at organization. As a direct result of these defects the gill tufts upon the affected side, normally dependent for their size chiefly upon the presence of these blood vessels, owing to their almost complete absence are markedly dwarfed or completely absent.

There is a great difference in size between the mesenchymal mass upon the affected half when compared with that upon the unaffected half of the same segments. That upon the affected half is much smaller owing to the defective size, shape and number of its cellular elements. The affected cells retain their earlier, embryological, spherular features. Isolation of the individual cells is most marked. The absence of mitotic figures in these cells is characteristic. Development of them has been strongly inhibited. There is no indication, however, of an edematous condition which might be anticipated in view of the absence of small blood vessels, thereby bringing about a separation of these cells as was found to be the case where the heart was markedly affected when the whole ovum was rayed. Microscopic study of these mesenchymal cells shows a lack of features of specialization in their protoplasm.

It is significant that evidences of reparative reaction to the injury sustained by the cells are entirely wanting. Assuming that the position of the neural tube and of the notochord indicates the median plane of the body, the cross-sectional area of the affected hemi-segments is reduced to less than half that of the normal segments. The several factors responsible for this condition are to be found, as was

noted above, in the undifferentiated condition and lack of growth of the myomeres, the pharynx, the blood vessels, the otic and optic vesicles and in the connective tissue-forming cells themselves.

These specimens demonstrate, therefore, the possibility of the production of localized injury by means of X-ray energy. This localization is sharply focused upon certain regions but involves all cells within the rayed area. This may be marked out most distinctly from the unaffected area. It is significant, in addition, that the injury received by the ovum at the two-cell stage should be transmitted through so many cell generations without any greater manifest effort at restitution. Sharp localization of organ-forming substances at this early stage of development is brought out as well by the experiments. The nature of the change whether physical or chemical is of less importance from the embryological standpoint than is the demonstration of symmetrical distribution of these substances at so early a developmental stage. A consideration of the nature of the change wrought, whether an upset in the phase of an emulsion globule or a distinct oxidation or reduction of protein, lipin or carbohydrate is less striking than the phenomenon of the transmission and distribution to subsequent cell-generations of this same altered substance. Here again this feature is overshadowed by the manifest inability either of the cells or of the organism as a whole to rectify the damage done. The absence of cell extrusions, of protoplasmic and of nuclear detritus, all argue for an invariably uniform quantity of energy applied to the embryos, and so regulated as to avoid these evidences of more severe injury as were noted in the previous paper. The absence of blood-cellular features of inflammatory reaction may be referred to two possible sources, due either to a destruction of the anlagen of these elements but for which no definite evidence exists, however, or because of the intra-cellular and non-destructive character of the alteration produced.

# THE RELATION OF THE EPINEPHRIN OUTPUT OF THE ADRENALS TO CHANGES IN THE RATE OF THE DENERVATED HEART

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## INTRODUCTION

It is well known that adrenalin causes acceleration of the heart after section of the vagi and excision of the stellate ganglia. v. Anrep (1) showed in the dog that stimulation of the peripheral end of the splanchnic nerve caused acceleration, associated with the peculiar features of the splanchnic blood pressure curve which Elliott (2) explains as due to the augmented epinephrin output known to be elicited by stimulation of that nerve. Pearlman and Vincent (3), working with the heart only partially isolated from the central nervous system by section of the vagi, have confirmed Elliott's interpretation of the peculiar form of the blood pressure curve caused by splanchnic stimulation and observed manifest augmentation of the heart. We have ourselves shown (in the cat) that the epinephrin given off at the normal average rate under the conditions of our experiments causes acceleration when the adrenal vein blood collected in a cava pocket for a minute or two is released. The acceleration begins a little time after release of the pocket, the length of the interval depending upon the state of the circulation, and is coincident or nearly so with the dilatation of the pupil if the superior cervical ganglion has been previously excised. The beginning of the dilatation has been seen to coincide approximately with the "dip" associated by Elliott with the action of the liberated epinephrin (4). Also when the splanchnic is stimulated with the adrenal veins open it can sometimes be seen that about the same time as the reactions of the eye (sensitized by removal of the superior cervical ganglion) appear, the denervated heart begins to become accelerated. From all this it seems clear that epinephrin

liberated from the adrenal in response to stimulation of the splanchnic plays a part in the acceleration of the heart. We shall show later on that the whole acceleration is not due to the epinephrin, but that other factors are involved, since a good acceleration, even as great as that obtained with intact adrenals, can be observed when they have been removed or when discharge of epinephrin has been prevented in other ways. It will be pointed out in discussing these results that, with such a reaction as acceleration of the heart, there is no inconsistency in attributing a share in the reaction to epinephrin and yet asserting that sometimes as great a maximum acceleration may be attained in its absence as when it is being given off. It is possible that the position of the maximum acceleration or of the beginning of the acceleration on the blood pressure curve may be shifted when epinephrin action is excluded. But however this may be, it will, we believe, be evident when our results have been displayed that it would be a very unpromising venture to attempt to found a method of estimating the rate of output of epinephrin upon such a reaction, even under conditions, as in splanchnic stimulation, in which it is known that an increase in the epinephrin output can contribute to it. Where adrenal blood is collected in a cava pocket and then released there is no question that the acceleration is produced by the epinephrin and practically by that alone, because the experiment has been so simplified that only one factor is acting, namely the admission into the circulation of the epinephrin-containing blood. But even here the reaction is of such a character that it could hardly lend itself to anything like an exact assay of the epinephrin in the blood from the pocket.

Nevertheless in a recent paper Cannon (5) has described experiments on cats in which, from the acceleration of the so-called denervated heart caused by stimulation of the central end of the sciatic nerve, by asphyxia and by emotional excitement, he professes to prove that these conditions produce a marked increase in the rate of output of epinephrin from the adrenals. Apparently admitting that the catheter method (6) is a difficult one to obtain positive results with, he introduces this as a relatively simple method which can be carried out by "any competent experimenter," and he states that the results of these experiments confirm "in every particular" his previous conclusions as to the influence of emotional excitement, asphyxia and sensory stimulation upon the adrenal secretion. We have never quarreled with the catheter method because of its difficulty, but because it cannot yield the data necessary to determine the rate of output of epinephrin or to

measure the changes in that rate. We shall take another occasion to point out again the reasons why we cannot accept Cannon's conclusions based on experiments with the catheter method. The technique of obtaining the cava blood is surely not beyond the reach of "any competent experimenter" in physiology, and the assaying of the epinephrin in the blood would be easy if such concentrations existed there as, by implication, we must conclude that Cannon assumed to exist during or after the action of the factors studied by him.

The denervated heart reaction now adopted by Cannon "as an indicator of adrenal secretion" is also easy enough to carry out. But it labors under even more serious defects, when employed as a quantitative method of measuring the epinephrin output and of estimating changes in the rate of output, than does the catheter method. For in the latter an attempt was at any rate made to obtain blood containing a portion of the epinephrin given off by the adrenals and to test it by a method of bio-assay which, if properly applied, does permit the epinephrin concentration in the sample of blood to be estimated. When Doctor Cannon stimulates the central end of the sciatic numerous reflex effects may be caused, the consequences of which upon the rate of the heart cannot be easily controlled. Among these the vasomotor reflex changes are conspicuous, and it is obvious that in this way great variations may be produced in the pressure in the cavities of the heart, the rate of blood flow through the coronary system and, therefore, the amount of epinephrin passing through the coronaries, any of which may lead to an acceleration of the heart beat without any change having occurred in the rate of output of epinephrin.

Cannon, however, states that after removal of the adrenals or their ligation *en masse* or after removal of one adrenal and section of the splanchnic of the other side, acceleration of the heart is no longer caused by sciatic stimulation. In one experiment he obtained some acceleration after division of both splanchnics in the thorax, but much less than before. He concludes that the acceleration following sciatic stimulation is due entirely to a reflexly increased output of epinephrin. And obviously assuming that no other factors are involved, he states that "comparisons of the increased rate due to sciatic stimulation with effects of adrenalin (quantitated as base) injected intravenously indicate that the range of reflex adrenal secretion lies between 0.001 and 0.005 mgm. per kgm. per minute, i.e., from 5 to 25 times the amount regarded by Stewart and Rogoff as the normal output." In view of our own results proving conclusively that epinephrin, if a factor, cannot

be the sole factor in the heart reaction elicited by stimulation of the sciatic, it would scarcely be worth while to spend much time in examining such data. But it may be pointed out that Cannon does not state by what control experiments he has established a quantitative relation between the *maximum* acceleration reached and the dose of adrenalin, and not, for instance, between the dose of adrenalin and the duration of the acceleration or the total surplus number of beats in the period of acceleration. Also if such data are to have real quantitative value the adrenalin ought to be administered while the blood pressure and therefore the coronary blood flow are increased to approximately the same extent as during stimulation of the sciatic.

As regards Cannon's statement that the reaction is abolished by removal of the adrenals, it must be noted that, if it were granted that the only change caused by removal of the adrenals or section of the splanchnics is the suppression of the epinephrin output, his result would simply show that the acceleration previously obtained had been due essentially to epinephrin. It would not show that any increase had occurred in the rate of output, unless it were demonstrated that a redistribution of the blood, due to the vascular reactions evoked by the stimulation and necessarily associated with the passage through the coronary circulation of an increased proportion of the epinephrin already being given off, was insufficient to account for the reaction.

But it cannot be granted that the operations practised to eliminate the epinephrin output have no other consequences. It is astonishing with what indifference both splanchnics are cut merely in order to interfere with the epinephrin output, as if all or most of the splanchnic fibers innervated the adrenals. The same is true of the removal of the adrenals. Some writers seem to assume that it is practically impossible to injure any important nerves when the adrenals are removed or tied off and that all the consequences which follow their removal are necessarily due to the loss of epinephrin. Gley and Quinquaud (7) have pointed out that the opposite is the case. In the dog according to them it is practically impossible to remove the adrenals without severely injuring the splanchnics. They say, however, that with their large experience they have succeeded in operating on the dog also, so as to eliminate the adrenals without injury to the splanchnics. Pearlman and Vincent (3) take exception to Gley's statement that the difference in the splanchnic blood pressure curve observed by v. Anrep (1) in dogs after and before removal of the adrenals is due to injury to nerve fibers, and no doubt in Vincent's hands the operation is as little harmful as it

is possible for it to be. If the operation can be more easily done in the cat, it still needs care and experience to reduce this cause of error to a minimum, particularly in the case of the right adrenal, the ligation of which is liable to injure the splanchnic. For this and other reasons the removal of the adrenals, as frequently done in acute experiments, may be attended with a marked drop in the blood pressure not related to loss of function of the glands. It must be remembered that in experiments on the denervated heart, the adrenals are removed after a considerable preliminary operation and the extrinsic regulatory nerves of the heart are eliminated. This may make it more difficult to excise or tie off the adrenals without a fall of blood pressure than in experiments on normal animals, such as those of Young and Lehman (8), of Hoskins and McClure (9) and of Bazett (10). The diminution in the heart rate remarked by Cannon, and apparently attributed by him entirely to the loss of epinephrin, is according to our observations not unrelated to the fall of pressure, although since there is reason to believe that the epinephrin liberated at the ordinary rate may affect the heart, some part of the slowing may be due to loss of epinephrin. Cannon has not given any data by which one can judge how great the change of blood pressure was in his experiments, but as he injected gum salt solution in one of them the fall may be assumed to have been sometimes considerable. Now, whatever interpretation one chooses to put upon the heart acceleration produced by sciatic stimulation, a reflex or more than one reflex action must be essentially involved in it. Any operation which impairs the conductivity of the reflex arcs must, therefore, tend to diminish or abolish the effect. And if a negative result obtained after removal of a certain organ is attributed solely to the specific effect which the operator intended to produce, and not at all to the general effects which he did produce, then, of course, positive results elicited before the operation in any way whatever will seem to depend entirely upon the specific activity of the organ removed.

In what has been said above we desire to state distinctly that we do not imply that Doctor Cannon did not remove the adrenals skilfully, and with full knowledge of the importance for his control experiment of injuring the nerves in the vicinity as little as possible and of maintaining the animal in a good general condition. All we know is that we obtained positive results after elimination of the adrenals where he obtained completely negative results. It is clearly the positive results which are significant for the decision of the question of the relation of



the epinephrin output to the heart acceleration caused by sciatic stimulation and not the negative ones.

Our experiments were all made on cats. The greater number of them were performed two years ago. Only a brief notice of a portion of them has been published (11). They were mentioned, so far as could be done in the few minutes allotted to us, in the discussion of Doctor Cannon's paper at the meeting of the American Physiological Society last December. We studied the effect upon the acceleration produced by stimulation of sensory nerves of eliminating the epinephrin secretion:

a. By clipping the adrenal veins, either with or without simultaneous ligation of the renal vessels.

b. By removing one adrenal (the right) and denervating the other and allowing the animal to recover completely from the operation. In a number of these animals the denervated adrenal was also removed in the final experiment, and the heart reactions obtained before and after its removal compared.

c. By removing one adrenal (almost always the right) and allowing an interval for the animal to recover, before performing the experiment upon sensory nerve stimulation with removal of the other adrenal. In this way it was supposed that the condition of the animal after removal of the remaining adrenal would be better than if both were removed at the time of the experiment.

d. By removing both adrenals during the experiment on the heart reaction. In all cases in excising the glands every precaution was taken to avoid injury to nerves, by making a careful dissection between the capsule of the gland and the cortex, tying the vessels with fine ligatures.

#### EXPERIMENTS IN WHICH THE ADRENAL VEINS WERE CLIPPED OFF OR TIED

In principle this is the most satisfactory way of eliminating the epinephrin output, since no other organ than the adrenals is interfered with and there is no damage to important nerves and no injurious fall of blood pressure. The procedure has been extensively employed by Gley. v. Anrep (1) also used it in some of his experiments on splanchnic stimulation and satisfied himself that the effects attributed by him to epinephrin were not obtained when the suprarenal vein was clipped and the corresponding splanchnic stimulated. And Pearlman and Vincent (3) state that they have usually obtained quite satisfactory

results by simply clamping and unclamping the adrenal veins. Cannon takes exception to clipping because it does not eliminate the heart reaction to sciatic stimulation or asphyxia which he interprets as indicating increased epinephrin output, and he, therefore, assumes that there must be leakage of epinephrin by anastomotic venous channels. He quotes, for instance, an experiment in which before ligation of the adrenal veins asphyxia of a certain duration caused an acceleration in the heart rate of 40 beats. After ligation of the veins the acceleration was precisely the same. We should have thought the only possible interpretation of such a result would have been that epinephrin had nothing to do with the reaction, in the particular experiment, at any rate. For who will believe that after the adrenal veins were tied just as much epinephrin passed out of the adrenals by some difficult collateral path, in the minute for which asphyxia was maintained, as would have passed out by the adrenal veins plus these hypothetical anastomotic channels? And how can the heart acceleration be a quantitative reaction for epinephrin if blocking the adrenal veins does not at least diminish the reaction? When a reaction known to be caused by epinephrin is studied the result is quite different. For example, we sometimes observe a small dilatation of the pupil (after previous removal of the superior cervical ganglion) on stimulation of the peripheral end of a splanchnic nerve, with the corresponding or even with both adrenal veins clipped. But this is much smaller than the reaction obtained before or after by similar stimulation with the veins open, and usually on release of the clips there is an additional and greater reaction indicating that epinephrin had been pent up in the adrenal veins by the clips. We suggest that the reason why Cannon gets such positive results with the adrenal veins tied, and must have recourse to removal or ligation of the adrenals to obtain negative results, is that in tying the veins he does not inflict such injury as causes a general deterioration of the animal incompatible with a positive heart reaction, whereas in removing the adrenals he appears to do so.

In our experiments with clipping of the adrenal veins we compared the heart acceleration and rise of blood pressure caused by stimulation of the sciatic when the blood flow from the glands and, therefore, the epinephrin output were going on unhindered, with the acceleration and blood pressure rise obtained when the flow from one or both adrenals was obstructed. It was supposed that if the epinephrin is an important factor in the heart reaction, the reaction would be distinctly

smaller with the adrenal veins clipped off, that is to say, provided that the reaction can be used at all as a quantitative test for epinephrin. The protocols show that this expectation was not realized. If the epinephrin liberated from the adrenals is an appreciable factor, it is not easy to disentangle its influence from that of the other factors which can affect the heart rate. It must be remembered that even if it were clearly demonstrated that the acceleration caused by sciatic stimulation was diminished by interference with the output of epinephrin, the other potential factors, such as rise of blood pressure, not being interfered with, this would only prove that epinephrin takes a share in the reaction, not that its rate of output is increased by the stimulation.

The peripheral end of a splanchnic nerve was also stimulated with the corresponding, or both adrenal veins clipped or open, in order to compare the effect of a procedure which is known to increase the epinephrin output on the heart rate with the effect of sciatic stimulation.

It will be seen that it may be difficult to demonstrate, by comparing the maximum acceleration caused with the adrenal veins clipped and open, that the epinephrin undoubtedly liberated by stimulation of the splanchnic takes any sensible share in the heart reaction. However, this is rather an illustration of the deficiencies of such a reaction as a quantitative test for epinephrin than a proof that the epinephrin liberated with the adrenal veins open is without effect upon the rate of the heart. As already mentioned, when the epinephrin is allowed to accumulate in a cava pocket or even when the epinephrin pent up in the adrenal vessels by clipping of the adrenal veins is released a distinct acceleration is produced, and it may be assumed that the epinephrin coming steadily off from the adrenals, without being accumulated, will tend to exert a similar action, especially when the amount of epinephrin passing per unit of time through the coronaries, or its concentration, is abruptly increased by the vasomotor changes associated with splanchnic or sciatic stimulation. It seems, however, improbable that with such a reaction as acceleration of the heart the acceleration produced by simultaneous action of two factors, singly effective, should be the sum of the separate effects, whether the reaction is measured by the maximum acceleration attained, or by the duration of the acceleration or by the total surplus number of beats. It seems more likely that when the heart is keyed up to a certain point by the action of one factor, whether this acts upon a local accelerator mechanism or not, it will break loose, so to speak, from the relative stability of rate

imposed upon it by removal of its extrinsic nerves, and execute a run of quicker beats, the duration and maximum acceleration of which may have no simple relation to the absolute magnitude of the exciting influence, and which may not be greatly modified by a concomitantly acting influence, itself capable of independently producing a similar reaction. The results of these experiments are illustrated by some protocols. Where it is mentioned that a nerve was stimulated with a vein clipped, the clip was applied, unless otherwise stated, a few seconds before the stimulation and was removed as soon as the portion of the curve to be used for counting the heart rate had been completed. When it is simply mentioned that a nerve was stimulated, without any reference to clipping, it is implied that the veins were open. Under "rate" is always given the number of heart beats per minute; under "pressure" the blood pressure in millimeters of mercury.

*Protocol.* Cat 175; male; weight, 2.2 kgm.

11:30 a.m. Urethane, 5 gm. by stomach tube.

1:10-1:48 p.m. Cut vago-sympathetics and excised stellate ganglia;<sup>1</sup> prepared central end of left sciatic for stimulation.

	<i>Rate</i>
1:50 p.m. Before stimulation of sciatic.....	188
25 seconds after beginning stimulation.....	210
1:55 p.m. Before stimulation of sciatic.....	187
20 seconds after beginning stimulation.....	202
40 seconds after beginning stimulation.....	212
Now opened abdomen and purposely manipulated intestines	
2:03 p.m. Before stimulation of sciatic.....	178
30 seconds after beginning stimulation.....	212
2:10 p.m. Before stimulation of sciatic.....	186
30 seconds after beginning stimulation.....	214
2:14 p.m. Before stimulation of sciatic; left adrenal vein clipped.....	184
30 seconds after beginning stimulation.....	206
2:16 p.m. Prepared peripheral end of left splanchnic in abdomen	
2:20 p.m. Before stimulation of splanchnic; left adrenal vein clipped....	184
15 seconds after beginning stimulation.....	192
30 seconds after beginning stimulation.....	208
45 seconds after beginning stimulation.....	224

<sup>1</sup> With the cat in the supine position an incision is made in the axilla and a part of the second rib exposed by separation of overlying muscle. A portion of the rib anterior to the angle is carefully separated from its periosteum and resected. The lower part of the stellate ganglion is usually thus exposed and the excision of the ganglion easily completed without injury to the pleura.

In all the experiments, except when otherwise stated, the period of stimulation of the sciatic was 30 to 40 seconds. When the heart rate is given after a certain number of seconds from beginning of stimulation this means that a sufficient portion of the curve beginning at that point was counted. The corresponding blood pressure was that at the end of the portion counted.

2:26 p.m.	Before stimulation of splanchnic; left adrenal vein clipped....	180	
	15 seconds after beginning stimulation.....	196	
	30 seconds after beginning stimulation.....	208	
	45 seconds after beginning stimulation.....	220	
	60 seconds after beginning stimulation.....	220	
2:32 p.m.	Before stimulation of splanchnic.....	182	
	During first 15 seconds of stimulation.....	180	
	During second 15 seconds of stimulation.....	200	
	During third 15 seconds of stimulation.....	204	
	During fourth 15 seconds of stimulation.....	216	
2:47 p.m.	Before stimulation of sciatic; both adrenal veins clipped.....	184	
	During first 15 seconds of stimulation.....	188	
	During second 15 seconds of stimulation.....	188	
	During third 15 seconds of stimulation.....	200	
	During fourth 15 seconds of stimulation.....	204	
2:53 p.m.	Before stimulation of sciatic.....	176	
	During first 15 seconds of stimulation.....	180	
	During second 15 seconds of stimulation.....	196	
	During third 15 seconds of stimulation.....	208	
	During fourth 15 seconds of stimulation.....	216	
3:05 p.m.	Before stimulation of sciatic; both adrenal veins clipped.....	188	
	During first 15 seconds of stimulation.....	188	
	During second 15 seconds of stimulation.....	196	
	During third 15 seconds of stimulation.....	204	
	During fourth 15 seconds of stimulation.....	204	
		<i>Rate</i>	<i>Pressure</i>
3:45 p.m.	Before stimulation of sciatic.....	176	82
	24 seconds after beginning stimulation.....	203	140
3:50 p.m.	Before stimulation of splanchnic; left adrenal vein clipped.....	174	58
	28 seconds after beginning stimulation.....	215	166
3:57 p.m.	Before stimulation of splanchnic; both adrenal veins clipped.....	185	62
	24 seconds after beginning stimulation.....	206	131
	40 seconds after beginning stimulation.....	221	
4:03 p.m.	Before stimulation of sciatic.....	174	46
	12 seconds after beginning stimulation.....	197	118
	40 seconds after beginning stimulation.....	200	
4:10 p.m.	Before stimulation of sciatic; both adrenal veins clipped.....	189	52
	15 seconds after beginning stimulation.....	200	112
	30 seconds after beginning stimulation.....	204	
4:15 p.m.	Before stimulation of splanchnic.....	184	55
	20 seconds after beginning stimulation.....	209	130
	Excised left adrenal		
4:30 p.m.	Before stimulation of splanchnic.....	215	70
	22 seconds after beginning stimulation.....	229	90

Some of the curves from these experiments are used in the next paper to demonstrate that the acceleration of the heart on stimulation of the central end of the sciatic, contrary to Cannon's statement, is easily obtainable after the abdomen has been opened. The protocols themselves and other protocols coming later in the present paper also afford abundant evidence that the statement is baseless. In figure 1 portions of the blood pressure curve from cat 175 before and during stimulation of the left splanchnic (*A* and *B*, taken at 3:50 p.m.) are

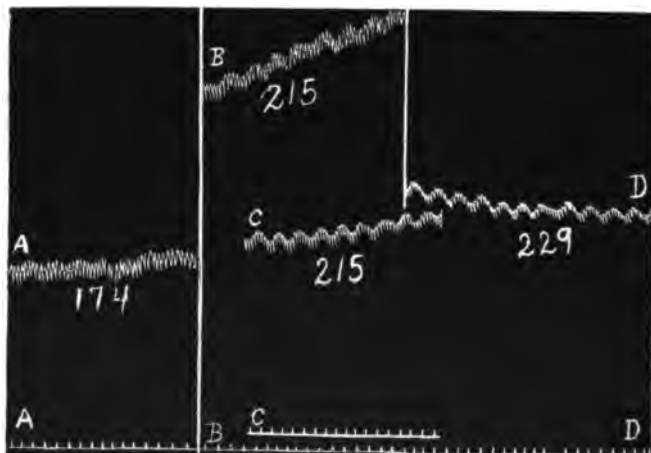


Fig. 1. Parts of blood pressure tracings from cat 175. *A*, before and *B*, a portion commencing 28 seconds after beginning of splanchnic stimulation with corresponding adrenal vein clipped; *C*, before and *D*, 22 seconds after beginning of splanchnic stimulation with corresponding adrenal excised. In all figures line of zero pressure corresponds with time trace; time in seconds; numbers above time trace represent heart rate per minute. Reduced to four-fifths.

reproduced, and corresponding portions after removal of the adrenal (*C* and *D*, taken at 4:30 p.m.). The maximum acceleration in *B* was 41 beats per minute; the maximum in *D*, 12 to 14 beats as compared with a maximum acceleration of 25 beats in the curve taken with the last splanchnic stimulation prior to removal of the adrenal. It is impossible to know how much, if any part, of the difference is due to the lack of epinephrin from the left adrenal when the curve *C* *D* was being written, since the stimulation was much less effective in raising the blood pressure, as shown in figure 2. The much reduced curves (black on white) are not intended for counting.

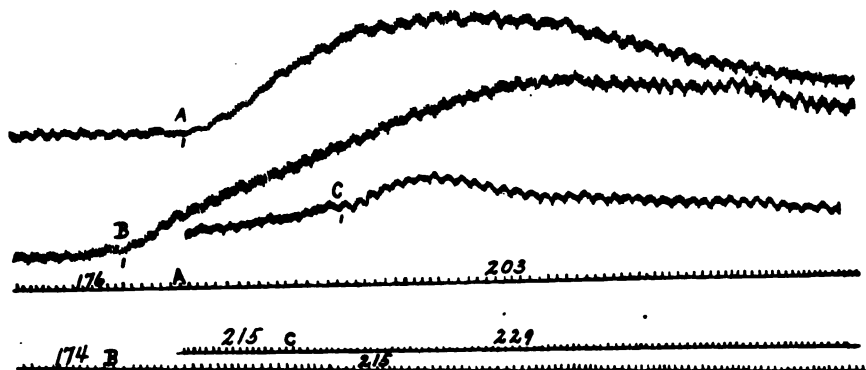


Fig. 2. Blood pressure curves from cat 175. A, sciatic stimulation; B, splanchnic stimulation with corresponding adrenal vein clipped; C, splanchnic stimulation with corresponding adrenal excised, 40 minutes after B. Reduced to one-half.

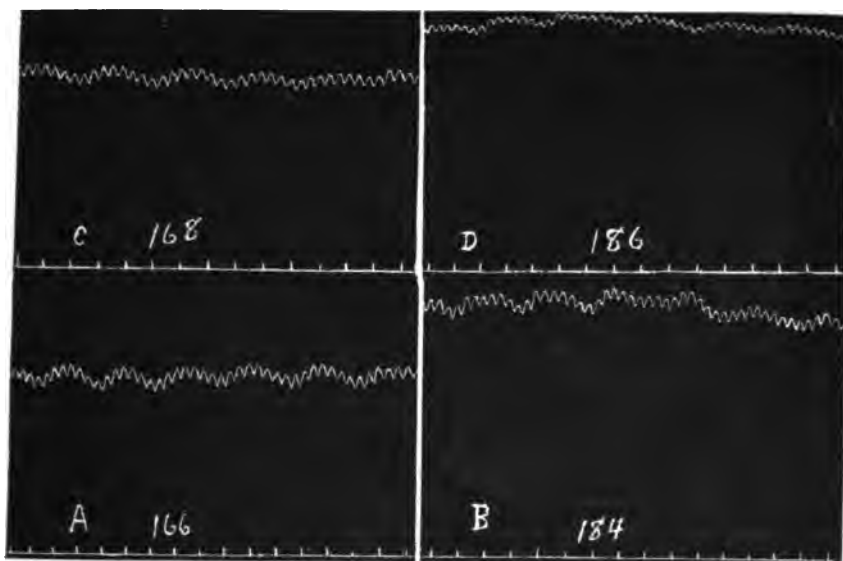


Fig. 3. Parts of blood pressure tracings from cat 237. A, before and B, a portion commencing 33 seconds after beginning of sciatic stimulation; C, before and D, 40 seconds after beginning of sciatic stimulation with both adrenal veins clipped. Reduced to three-fourths.

In figure 3 portions of the curve from cat 237 are given, *A* and *B* taken respectively before and during stimulation of the sciatic with the adrenal veins open and *C* and *D* with the adrenal veins clipped. The curves were practically parallel and the acceleration of the heart about the same in both (about 18 beats per minute), in the portions shown in the figure. The maximum acceleration with stimulation after clipping was 21, and without clipping 20 beats per minute. It may be remarked that not only had the abdomen been opened but the renal arteries and veins had been tied 40 minutes before these curves were obtained.

*Protocol.* Cat 177; male; weight, 3.6 kgm.

2:05 p.m. Under urethane (6 gm.) cut vago-sympathetics; excised stellate ganglia and prepared central end of left sciatic for stimulation.

		<i>Rate</i>	<i>Pressure</i>
2:25 p.m.	Before sciatic stimulation.....	185	132
	20 seconds after beginning stimulation.....	228	154
2:30 p.m.	Opened abdomen; prepared peripheral end of left splanchnic		
2:32 p.m.	Before sciatic stimulation.....	173	105
	24 seconds after beginning stimulation.....	200	128
	49 seconds after beginning stimulation.....	216	
2:35 p.m.	Before stimulation of left splanchnic; left adrenal vein clipped.....	167	108
	12 seconds after beginning stimulation.....	191	
	24 seconds after beginning stimulation.....	215	140
	45 seconds after beginning stimulation.....	211	110
2:40 p.m.	Before stimulation of sciatic; both adrenal veins clipped.....	181	80
	23 seconds after beginning stimulation.....	190	
	37 seconds after beginning stimulation.....	197	106
2:45 p.m.	Before stimulation of left splanchnic; left adrenal vein clipped.....	171	90
	17 seconds after beginning stimulation.....	194	
	22 seconds after beginning stimulation.....	206	124
2:50 p.m.	Before stimulation of left splanchnic; both adrenal veins clipped.....	191	100
	20 seconds after beginning stimulation.....	213	128
2:55 p.m.	Before stimulation of sciatic; left adrenal vein clipped..	184	94
	28 seconds after beginning of stimulation.....	192	107
3:00 p.m.	Before stimulation of sciatic; both adrenal veins clipped.....	167	78
	23 seconds after beginning stimulation.....	173	98
3:10 p.m.	Before stimulation of left splanchnic; left adrenal vein, clipped.....	160	74
	18 seconds after beginning stimulation.....	194	92



3:15 p.m.	Before stimulation of left splanchnic; both adrenal veins clipped.....	160	82
	23 seconds after beginning stimulation.....	167	
	42 seconds after beginning stimulation.....	185	
	55 seconds after beginning stimulation.....	200	106
3:20 p.m.	Before stimulation of left splanchnic; left adrenal vein clipped.....	165	72
	20 seconds after beginning stimulation.....	183	82
3:30 p.m.	Before stimulation of left splanchnic; both adrenal veins clipped.....	166	68
	25 seconds after beginning stimulation.....	186	86
3:35 p.m.	Before stimulation of left splanchnic; left adrenal vein clipped.....	173	76
	20 seconds after beginning stimulation.....	180	90
4:10 p.m.	Before stimulation of peripheral end of right splanchnic in thorax, with left adrenal vein clipped.....	170	30
	15 seconds after beginning stimulation.....	205	78

In cat 177, in which a good acceleration of the heart was obtained with sciatic stimulation after opening the abdomen and section of one splanchnic nerve, the reaction from the sciatic after repeated splanchnic and sciatic stimulations and repeated clipping of the adrenal veins was distinctly diminished toward the end of the experiment. Stimulation of the peripheral end of the splanchnic continued to give a good acceleration of the heart when the reaction, as reflexly elicited by stimulation of the central end of the sciatic, together with the reflex rise of blood pressure, was being exhausted.

In comparing the acceleration accompanying a given rise of blood pressure caused by sciatic, with that accompanying a similar rise of pressure caused by splanchnic stimulation it generally appeared that the splanchnic acceleration was the greater. This, of course, would be consistent with the view that the epinephrin secretion in response to direct splanchnic stimulation may play a substantial part in the reaction. It would appear probable that any share of the epinephrin in the acceleration both when the output is increased, as by splanchnic stimulation, and when, without an actual increase in the output, more epinephrin is sent through the coronary circulation in response to a rise of blood pressure caused by sensory nerve stimulation, must vary with the state of the heart and may, therefore, be expected to vary in different animals and in the same animal at different periods of an experiment.

*Protocol.* Cat 179; male; weight, 2.2 kgm. Under urethane (5 gm.) cut vago-sympathetics; excised stellate ganglia; prepared central end of left sciatic for stimulation.

		<i>Rate</i>	<i>Pressure</i>
4:00 p.m.	Before sciatic stimulation.....	169	80
	25 seconds after beginning stimulation.....	210	130
4:05 p.m.	Prepared peripheral end of left splanchnic (extraperitoneally)		
4:10 p.m.	Before stimulation of left splanchnic; left adrenal vein clipped.....	168	70
	20 seconds after beginning stimulation.....	182	94
4:13 p.m.	Before sciatic stimulation.....	173	81
	32 seconds after beginning stimulation.....	204	113
4:17 p.m.	Before stimulation of left splanchnic; left adrenal vein clipped.....	177	73
	20 seconds after beginning stimulation.....	180	98
4:20 p.m.	Before stimulation of left splanchnic.....	170	80
	24 seconds after beginning stimulation.....	183	109
4:25 p.m.	Before stimulation of sciatic; left adrenal vein clipped..	170	76
	23 seconds after beginning stimulation.....	180	106
4:28 p.m.	Before stimulation of left splanchnic; left adrenal vein clipped.....	170	71
	23 seconds after beginning stimulation.....	180	100
4:30 p.m.	Opened abdomen; manipulated intestines		
4:32 p.m.	Before sciatic stimulation.....	171	66
	25 seconds after beginning stimulation.....	196	102
4:35 p.m.	Before stimulation of sciatic; both adrenal veins clipped.....	173	64
	20 seconds after beginning stimulation.....	192	95
4:40 p.m.	Before stimulation of left splanchnic with both adrenal veins clipped.....	173	70
	21 seconds after beginning stimulation.....	179	103
4:45 p.m.	Before stimulation of splanchnic.....	171	62
	25 seconds after beginning stimulation.....	179	90
4:48 p.m.	Cut right splanchnic		
4:50 p.m.	Before sciatic stimulation.....	160	44
	23 seconds after beginning stimulation.....	161	56

The protocol of cat 179 shows again quite clearly good heart reactions with sciatic stimulation after opening the abdomen and section of one splanchnic. The practical disappearance of the reaction after section of the remaining splanchnic, when the blood pressure fell to 44 mm. of mercury is well shown. . But is it likely that this disappearance is due solely to the suppression of the epinephrin secretion when a little earlier in the experiment a decided reaction was obtained by stimulation of the sciatic with both adrenal veins clipped and after section of the left splanchnic? Here the blood pressure reaction was a

good one, the pressure rising from 64 to 95 mm. of mercury. But after the section of the second splanchnic only a trifling rise of pressure was caused by sciatic stimulation. The absence of any substantial rise of pressure and the depressing influence of the lowered blood pressure on the paths for any other reflexes than the vascular reflexes which may be concerned in the reaction offer a more probable explanation.

In a number of cats one superior cervical ganglion (the left) had been previously excised, so that it was possible to compare the acceleration of the heart caused by stimulation of the sciatic or splanchnic with the eye reactions. The following is a typical protocol.

*Protocol.* Cat 190; female; weight, 2.31 kgm. Left superior cervical ganglion excised 21 days previously. Under urethane (5 gm.) cut vago-sympathetics; excised stellate ganglia; prepared central end of left sciatic for stimulation.

		<i>Rate</i>	<i>Pressure</i>
1:05 p.m.	Before stimulation of sciatic <sup>2</sup> .....	253	123
	35 seconds after beginning stimulation.....	259	164
1:10 p.m.	Before sciatic stimulation <sup>2</sup> .....	229	
	15 seconds after beginning stimulation.....	237	
1:15 p.m.	Prepared peripheral end of left splanchnic (extraperitoneally)		
1:35 p.m.	Before stimulation of left splanchnic.....	231	80
	10 seconds after beginning stimulation.....	268	155
	Very good pupil and nictitating reactions in 9 seconds		
1:40 p.m.	Before stimulation of left splanchnic with left adrenal vein clipped.....	223	64
	20 seconds after beginning stimulation.....	280	150
	Small pupil and nictitating reactions in 18.4 seconds		
1:50 p.m.	Before stimulation of left splanchnic with left adrenal vein clipped.....	227	70
	20 seconds after beginning stimulation.....	263	
	48 seconds after beginning stimulation.....	278	130
	Very small eye reactions in 24.8 seconds		
2:15 p.m.	Excised left adrenal		
2:20 p.m.	Before stimulation of left splanchnic.....	219	72
	25 seconds after beginning stimulation.....	227	100
	Doubtful, if any eye reactions		

<sup>2</sup> Both pupils dilate instantaneously, the left becoming much wider than the right, both returning to previous state very soon after stimulation of the sciatic is stopped. With a prolonged or strong stimulation the left nictitating may slowly retract. This reaction is distinctly different from that obtained with splanchnic stimulation (when the adrenal veins are free) which affects only the denervated eye and occurs after a distinct latent period.

2:30 p.m.	Before stimulation of sciatic.....	223	80
	22 seconds after beginning stimulation.....	262	168
2:35 p.m.	Opened abdomen; prepared peripheral end of right splanchnic		
2:40 p.m.	Before stimulation of right splanchnic.....	210	45
	20 seconds after beginning stimulation.....	285	105
2:55 p.m.	Before clipping aorta just above diaphragm.....	249	48
	After clipping aorta just above diaphragm.....	260	146

It is to be remarked that when the left splanchnic was stimulated with the left adrenal vein clipped, the eye reactions were not completely abolished but were much diminished and appeared after a much longer delay than when the splanchnic was stimulated with the vein open. Complete loss of the eye reactions on stimulating the peripheral end of a splanchnic nerve may be seen when the corresponding adrenal vein has been clipped off, although good reactions were being obtained with the vein open, or the result may be what was found in cat 190. This result is compatible with the view that some epinephrin may find its way into the blood stream by a collateral route, as suggested by Cow (12) (the renal vessels were not tied in this experiment\*) but may also be due solely to a change in the concentration or quantity of epinephrin from the other adrenal passing through the coronary circulation, associated with the vasomotor effects of the splanchnic stimulation. The observation that in this experiment after excision of the left adrenal stimulation of the left splanchnic produced only a doubtful, if any, pupil reaction does not enable us to decide against the latter view. For the vasomotor effect (rise of blood pressure) and the heart acceleration were also much reduced, possibly owing to some injury to the splanchnic in removal of the adrenal. The fact that the eye reactions, while still present, were greatly diminished by the clipping of the adrenal vein and that it took a much greater time for them to appear while the acceleration of the heart was not at all diminished, shows clearly that the latter reaction cannot be a quantitative test for epinephrin. With sciatic stimulation the pupil reaction is complicated by the fact that both pupils dilate immediately after stimulation, of course through the nervous system. Although the sensitized pupil widens more than the other, this reaction is different from the typical epinephrin reaction yielded, for instance, by splanchnic stimulation. The return of the pupils to their previous size on stoppage of the stim-

\*In making a cava pocket we generally tie the renal vein at the hilus and also at its entrance into the cava.

ulation begins at once and is accomplished more quickly after sciatic stimulation than when a true epinephrin reaction has been induced by splanchnic stimulation. Further, the pupil reaction elicited by stimulation of the sciatic after interference with the epinephrin output by removal of one adrenal and section of the nerves of the other, or after removal of both adrenals, has the same characters as that seen with intact adrenals. A nictitating reaction was not obtained with sciatic stimulations which caused marked acceleration of the heart. Only with quite strong stimulation of the sciatic was there any retraction of the nictitating. This also is different from the genuine epinephrin eye reactions. The observations on the eye reactions, then, are inconsistent with the idea that stimulation of the sciatic causes a marked increase in the output of epinephrin, which in its turn causes the observed acceleration of the heart. It may further be asked how an acceleration (see protocol of cat 190) caused by sciatic stimulation, which is much smaller with both splanchnics intact at the beginning of the experiment than later on when one splanchnic has been divided and the corresponding adrenal removed, can be considered a quantitative reaction for the rate of output of epinephrin. It will be seen in another section of the paper that it is common, or indeed the rule, to obtain as large a reaction on stimulation of the sciatic in animals from which one adrenal has been removed and the animal allowed to recover as in animals with both adrenals intact.

Another cat (191) from which one superior cervical ganglion had been previously removed yielded results so similar to those of cat 190 that the protocol need not be reproduced. The only difference was that sciatic stimulation, as usual, gave a good acceleration at the beginning of the experiment, from 240 to a maximum of 295 beats per minute, the blood pressure rising from 124 to 182 mm. of mercury. With a second stimulation of the sciatic the heart rate increased from 256 to 275, the blood pressure rising from 112 to 150 mm. The eye reactions were the same as in cat 190. The abdomen was now opened and the peripheral end of the left splanchnic stimulated. The pulse rate rose from 243 to 270 beats, the blood pressure from 90 to 144 mm. of mercury. Excellent pupil and nictitating reactions were obtained in 9 seconds. The left adrenal vein was then clipped and the left splanchnic again stimulated. The heart rate increased from 237 to 262 beats per minute and the blood pressure from 110 to 150 mm. of mercury. Small pupil and nictitating reactions were observed in 20.4 seconds.

*Protocol.* Cat 195; female; weight, 2.4 kgm. Left superior cervical ganglion excised 14 days previously. Under urethane (5.5 gm.) prepared peripheral end of left splanchnic for stimulation in abdomen, tied lumbar veins just before they cross the adrenals; prepared central end of left sciatic for stimulation.

		<i>Rate</i>	<i>Pressure</i>
2:55 p.m.	Before stimulation of left splanchnic.....	202	78
	12 seconds after beginning stimulation.....	216	96
	Very good pupil and nictitating reactions in 12.8 seconds		
3:00 p.m.	Before stimulation of left splanchnic; left adrenal vein clipped.....	186	80
	19 seconds after beginning stimulation.....	211	108
	No eye reactions		
3:05 p.m.	Before stimulation of left splanchnic; left adrenal vein clipped.....	178	71
	14 seconds after beginning stimulation.....	181	78
3:20 p.m.	Cut vago-sympathetics and excised stellate ganglia		
3:30 p.m.	Before stimulation of sciatic <sup>4</sup> .....	145	52
	20 seconds after beginning stimulation.....	169	94
3:35 p.m.	Before weaker stimulation of sciatic.....	152	63
	54 seconds after beginning stimulation.....	151	63
3:40 p.m.	Prepared peripheral end of right splanchnic in thorax		
3:42 p.m.	Before stimulation of right splanchnic.....	146	35
	40 seconds after beginning stimulation.....	192	76
	Good eye reactions in about 40 seconds		
3:45 p.m.	Before stimulation of right splanchnic; right adrenal vein clipped.....	157	44
	20 seconds after beginning stimulation.....	184	
	30 seconds after beginning stimulation.....	207	82
	No eye reactions		
3:50 p.m.	Before stimulation of right splanchnic; both adrenal veins clipped.....	150	44
	40 seconds after beginning stimulation.....	199	84
	No eye reactions		
3:55 p.m.	Before stimulation of right splanchnic.....	146	44
	30 seconds after beginning stimulation.....	200	68
	Good eye reactions occurred in 30-35 seconds		

In the above experiment (cat 195) the abdomen was opened, the lumbar vein tied just before it crosses the left adrenal and the left splanchnic stimulated several times before the vago-sympathetics were cut and the stellate ganglia excised. The central end of the sciatic was then stimulated and caused an acceleration of 24 beats and a rise of blood pressure from 52 to 94 mm. of mercury, showing again that Cannon's statement that the heart reaction is scarcely ever obtained with sciatic stimulation after opening the abdomen is unfounded.

<sup>4</sup> Eye reactions with sciatic stimulation as described in protocol of cat 190, p. 319.

Of course when a weak enough stimulus was employed (5 minutes thereafter) the heart rate remained unchanged, but so did the blood pressure. Precisely the same result was obtained with stimulation of the peripheral end of the splanchnic, a stimulus which failed to cause any appreciable rise of blood pressure (as at 3:05 p.m.) also caused little if any acceleration of the heart. Whether the corresponding adrenal vein was open or clipped seemed to have no influence on the maximum acceleration. Indeed in the first splanchnic stimulation (at 2:55 p.m.) with the vein open the maximum acceleration was only 14 beats, whereas in the next stimulation, with the vein clipped, it was 25 beats per minute. Yet the eye reactions, which were very good in the first case, were abolished in the second. The eye reactions are universally admitted to be due to epinephrin, when elicited by stimulation of the peripheral end of the splanchnic. How is it possible to believe that when they are negative, while the heart acceleration is even greater than when they were strongly positive, the acceleration of the heart is a specific reaction for epinephrin?

In another cat (196), in which the left superior cervical ganglion had been excised 20 days previously, the vago-sympathetics were cut, the stellate ganglia excised and the abdomen opened under urethane at the beginning of the experiment. The lumbar veins, just before crossing the adrenals, and the renal arteries and veins were tied on both sides and both adrenal veins prepared for clipping. The peripheral end of the left sympathetic in the thorax was prepared for stimulation and the central end of a sciatic. Stimulation of the left sympathetic with the adrenal veins open gave a good pupil reaction in 8 seconds. The heart rate, which was 212 before stimulation, reached a maximum of 255 beats per minute (counting from 16 seconds after beginning of stimulation). The blood pressure rose from 72 to 150 mm. of mercury. The left sympathetic was now stimulated with the left adrenal vein clipped. There was no pupil reaction. The heart rate increased from 201 to a maximum of 260 beats per minute (counting from 16 seconds after the beginning of stimulation) and the blood pressure from 94 to 134 mm. of mercury. Then the left sympathetic was again stimulated with both adrenal veins clipped. There was no pupil reaction but the heart rate increased from 200 to a maximum of 245 beats a minute and the blood pressure from 58 to 107 mm. of mercury. Is not this again quite inconsistent with the view that the heart acceleration constitutes a quantitative reaction by which the output of epinephrin can be determined?

The next protocol (from cat 200) illustrates the general parallelism between the acceleration of the heart elicited by sciatic stimulation and the rise of blood pressure, and the absence of any demonstrable influence of clipping of the adrenal veins on either the maximum acceleration or the increase of blood pressure.

*Protocol.* Cat 200; female; weight, 1.47 kgm. Left superior cervical ganglion excised 16 days previously. Under urethane anesthesia cut vago-sympathetics; excised stellate ganglia; prepared central end of sciatic for stimulation.

		<i>Rate</i>	<i>Pressure</i>
10:00 a.m.	Before sciatic stimulation <sup>5</sup> .....	210	84
	Counts of successive portions of the curve after beginning of stimulation (with progressive increase in strength).....	211 215 230 230 244	84 102 112 128 128
10:05 a.m.	Before sciatic stimulation.....	214	100
	Counts of successive portions of the curve with stimulation as above.....	224 240 240	117 126 118
10:10 a.m.	Prepared adrenal veins for clipping (extraperitoneally)		
10:15 a.m.	Before sciatic stimulation.....	217	84
	Successive counts after beginning of stimulation as above.....	223 234 248	100 110 110
10:20 a.m.	Before sciatic stimulation; both adrenal veins clipped.	206	70
	Successive counts after beginning of stimulation as above.....	212 222 223 225	88 89 88 86
	Just after release of adrenal veins.....	228	95
	15 seconds after release of adrenal veins.....	245	104
10:25 a.m.	Before sciatic stimulation.....	214	80
	Successive counts as above during stimulation.....	216 239 253	88 104 117
10:30 a.m.	Before sciatic stimulation; both adrenal veins clipped.	215	77
	During stimulation as above.....	227 228	84 98
	Just after release of adrenal veins.....	236	96
	12 seconds after release of adrenal veins.....	239	100

In the last experiment (cat 193) to be quoted in this section of the paper, only the vago-sympathetics were cut at the beginning of the experiment, and the abdomen was opened.

<sup>5</sup> The effects on the eye were those described in the footnote to protocol of cat 190, p. 319.



*Protocol.* Cat 193; male; weight, 2.7 kgm. Left superior cervical ganglion excised 10 days previously. Under urethane (5 gm.) opened abdomen; prepared peripheral end of left splanchnic for stimulation; cut vago-sympathetics.

	<i>Rate Pressure</i>	
12:08 p.m.	Before stimulation of splanchnic; left adrenal vein clipped.....	
	157	98
	11 seconds after beginning stimulation.....	
	175	
	18 seconds after beginning stimulation.....	
	180	145
	No eye reactions during stimulation; after release of clip good pupil and nictitating reactions in 9.2 seconds	
12:10 p.m.	Before stimulation of splanchnic .....	
	162	96
	10 seconds after beginning stimulation.....	
	209	136
	Good pupil and nictitating reactions in 11.5 seconds	
12:15 p.m.	Before stimulation of splanchnic; left adrenal vein clipped.....	
	172	120
	17 seconds after beginning stimulation.....	
	190	162
	No eye reactions during stimulation; on release of clip good pupil and nictitating reactions in 8.2 seconds	
12:20 p.m.	Excised stellate ganglia	
12:40 p.m.	Before stimulation of splanchnic; left adrenal vein clipped.....	
	185	114
	18 seconds after beginning stimulation.....	
	201	144
	No eye reactions during stimulation; on release of clip good pupil and nictitating reactions in 11 seconds	
12:50 p.m.	Stimulated splanchnic with left adrenal vein and coeliac and superior mesenteric arteries clipped.	
	Before stimulation.....	
	203	134
	17 seconds after beginning stimulation.....	
	202	136
	No eye reactions during stimulation; on release of adrenal vein good pupil and nictitating reactions in 10.6 seconds	
	Before removal of adrenal clip.....	
	202	121
	12 seconds after release of adrenal vein.....	
	238	128
	Removed clips from coeliac and superior mesenteric	
1:00 p.m.	Prepared central end of sciatic for stimulation	
1:02 p.m.	Before stimulation of sciatic.....	
	174	78
	11 seconds after beginning stimulation.....	
	184	102
	20 seconds after beginning stimulation.....	
	194	
	34 seconds after beginning stimulation.....	
	200	104
1:20 p.m.	Before stimulation of sciatic.....	
	184	68
	16 seconds after beginning stimulation.....	
	200	108

The effects of stimulating the splanchnic, with the corresponding adrenal vein open and clipped, were not obviously different from those in the other experiments, where the stellate ganglia had been excised at the beginning, nor did removal of the stellate ganglia cause any essential change. The failure of the heart reaction with splanchnic

stimulation when the coeliac and superior mesenteric arteries were clipped is clearly associated with the absence of a rise of pressure when the splanchnic area is thus eliminated. On removal of the clip from the adrenal vein the usual good eye reactions were obtained and also an acceleration of the heart due to release of the epinephrin pent up in the adrenal vessels.

It may be tedious to point out again that an hour and a half after the abdomen had been opened, with one splanchnic cut, after all the clipping and unclipping of the adrenal veins and of the coeliac and superior mesenteric arteries (and a good many observations actually made have been omitted from the protocol to save space) stimulation of the sciatic still gave a good acceleration of the heart, although, according to Cannon, no reaction ought to have been obtained. In view of such facts what becomes of Doctor Cannon's assertion, unsupported by any evidence that when we collect adrenal vein blood from a cava pocket, the "peculiar" conditions of our experiments do not permit of the demonstration that with stimulation of sensory nerves there is a vast outpouring of epinephrin from the adrenals, a demonstration which he obtains by a misinterpretation of a heart reaction equally well elicited whether the adrenal veins are open or clipped off, whether the eye (sensitized by removal of the superior cervical ganglion) is giving negative or positive reactions for epinephrin? Since, however, Doctor Cannon maintains that the mere clipping or tying of the adrenal veins need not interfere in the least with the passage of epinephrin to the heart, and has even convinced himself that adrenalin, injected into the lumbar vein crossing the adrenal after it has been ligated on each side of the gland, passes so freely into the circulation that it causes a large rise of blood pressure, evidence of another kind will now be given that the acceleration of the heart relied upon by him to prove a markedly augmented rate of epinephrin output when the sciatic nerve is stimulated has no such significance.

#### EXPERIMENTS ON ANIMALS WITH ONE ADRENAL REMOVED AND THE NERVES OF THE OTHER CUT

We have previously shown (13) that after this operation the epinephrin output is either greatly reduced or abolished, within the limits of sensitiveness of the test objects (rabbit's intestine and uterus segments) employed to detect and estimate it. It was therefore of interest to see whether the acceleration of the heart would

be obtained in these animals by stimulation of sensory nerves. The result was positive in all the cats, a good acceleration being elicited by stimulation of the sciatic. For example, in cat 201, a female weighing 2.575 kgm., the right adrenal was removed, the nerves of the left including the splanchnic cut. A portion of the left semilunar ganglion was removed. The left superior cervical ganglion was excised at the same time. Eighteen days thereafter, the animal being in good condition, the vago-sympathetics were cut and the stellate ganglia removed under urethane and the central end of the left sciatic nerve prepared for stimulation. A blood pressure tracing was taken as usual from the right carotid. Stimulation of the sciatic caused the heart rate to increase from 177 before stimulation to a maximum rate of 211 beats per minute. The blood pressure increased from 54 mm. before stimu-

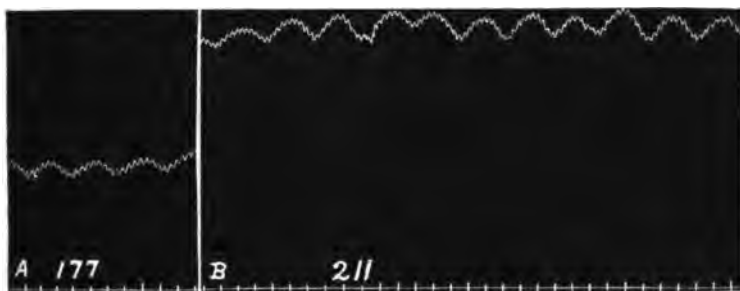


Fig. 4. Parts of blood pressure tracings from cat 201. A, before and B, a portion commencing 20 seconds after beginning of sciatic stimulation. Reduced to three-fifths.

lation to a maximum of 132 mm. of mercury. The acceleration and the blood pressure increased together. Successive portions of the curve starting from the point of stimulation yielded the following heart rates and blood pressures 180 (72), 193 (97), 202 (107), 209 (118), 209 (132). The blood pressures are in parentheses. A sample of the curve is reproduced in figure 4. The effect of sciatic stimulation on the pupils did not differ materially from that already described in cats in which the epinephrin output had not been interfered with (see footnote to protocol of cat 190). The nictitating membrane did not move. The abdomen was afterwards opened, a short cava pocket made and adrenal vein blood collected, which was assayed on rabbit segments. It was shown that the epinephrin output could not have been more than one-fiftieth of the average output per kilogram per minute, under

the conditions of our experiments. The preliminary operation had, therefore, effectively severed the epinephrin-secreting nerves of the left adrenal. In spite of this and also in spite of the fact that one splanchnic had been divided, and with both splanchnics intact a greater reflex rise of blood pressure might have been obtained, a maximum acceleration of the heart of 32 to 34 beats was elicited by stimulation of the sciatic. According to Cannon, the whole of the acceleration must have been due to a great outpouring of epinephrin from the left adrenal, reflexly stimulated along efferent nerve paths which had been divided 18 days before, and which certainly had not regenerated in that time.

In another cat (202), a female weighing 2.545 kgm., the right adrenal was removed and the nerves of the left cut. The left superior cervical ganglion was excised at the same time. Eighteen days thereafter the heart was denervated under urethane and blood pressure tracings taken from the right carotid. Stimulation of the central end of the sciatic caused an acceleration of the heart rate from 145 per minute before stimulation to 161. The blood pressure rose from 92 to 134 mm. of mercury. Successive portions of the curve, from the beginning of stimulation, yielded the following heart rates and blood pressures: 152 (100), 155 (110), 157 (130), 160 (126), 161 (134). The pupil reactions were as previously described in the footnote to the protocol of cat 190. There was no movement of the nictitating membrane. After the sciatic stimulations the abdomen was opened, and 3 specimens of adrenal vein blood collected—the first, about 1 gram (discarded), the second, 2.9 grams in 4 minutes (0.75 gm. per minute), the third 5.1 grams in 9 minutes (0.57 gm. per minute). No evidence was obtained with rabbit segments that the blood contained any epinephrin. It was shown that the third specimen could not have contained 1:70,000,000 epinephrin, i.e., the output could not have been 0.000007 mgm. per minute for the cat, or 0.0000027 mgm. per kilogram per minute. In other words it could not have been one-hundredth of the average normal output, and there was no evidence that any epinephrin was being given off. The interval after the preliminary operation was quite short in this animal, 8 days, which possibly may be an unfavorable condition for obtaining a large heart reaction, although we have no evidence as to this.

In the next experiment (cat 215) the animal was allowed to survive for 2 months.

*Protocol.* Cat 215; female; weight 2.38 kgm.

May 17, 1918. Right adrenal excised, left denervated and right superior cervical ganglion excised.

July 15, 1918. Under urethane (5 gm.) cut vago-sympathetics, excised stellate ganglia, prepared central end of left sciatic for stimulation.

		<i>Rate</i>	<i>Pressure</i>
11:15 a.m.	Before sciatic stimulation for 38 seconds (8 cm.) <sup>*</sup> .....	219	134
	During first 14 seconds of stimulation.....	232	162
	During next 13 seconds of stimulation.....	238	170
	During next 13 seconds.....	244	172
	During next 20 seconds.....	228	147
11:20 a.m.	Before sciatic stimulation for 33 seconds (6 cm.).....	222	144
	During first 14 seconds of stimulation.....	240	180
	During next 14 seconds of stimulation.....	248	196
	During next 15 seconds.....	249	180
11:25 a.m.	Before sciatic stimulation for 26 seconds (6.5 cm.).....	219	152
	During first 8 seconds of stimulation.....	226	178
	During next 7 seconds of stimulation.....	230	182
	During next 12 seconds.....	246	190
11:30 a.m.	Before sciatic stimulation for 40 seconds (6 cm.).....	224	148
	During first 13 seconds of stimulation.....	239	177
	During next 14 seconds of stimulation.....	252	180
	During next 13 seconds of stimulation.....	251	182
	During next 10 seconds.....	248	168
11:35 a.m.	Before sciatic stimulation for 37 seconds (4 cm.).....	222	148
	During first 12 seconds of stimulation.....	239	173
	During next 11 seconds of stimulation.....	244	180
	During next 11 seconds of stimulation.....	252	180
	During next 14 seconds.....	249	168

Later on the abdomen was opened and adrenal vein blood collected. Assays of the adrenal blood released from a cava pocket were also made by the pupil reaction.

The protocol shows that stimulation of the sciatic caused good acceleration of the heart with corresponding changes in the blood pressure. The rabbit intestine assay of the adrenal blood demonstrated that a substantial output of epinephrin was still going on. The concentration of the second specimen was about 1:6,500,000 and that of the third specimen about 1:3,750,000, corresponding in either case to an output of 0.00008 mgm. per minute for the cat, or 0.00003 mgm. per kilogram per minute, one-seventh or one-eighth of the average normal output. It is impossible to say whether an unusually large proportion of the secretory fibers of the left adrenal had escaped

<sup>\*</sup> Eye reactions, as described in footnote to protocol of cat 190, p. 319. The distance between the coils is given in brackets.

section at the preliminary operation, or whether some regeneration had occurred. In any case the innervation of the one adrenal remaining must still have been seriously crippled. Yet the heart reaction on which Cannon relies as an "indicator of adrenal secretion" was as well obtained with sciatic stimulation as in normal animals, corresponding with the general excellent condition of the cat two months after the primary operation.

In two cats operated on in the same way but only a relatively short time before (8 days and 7 days), the experiment was completed by excising the remaining (already denervated) adrenal. It was not thought advisable in these cats to complicate the experiment by attempting to estimate the residual epinephrin output, if any, on the adrenal vein blood. But it was shown that the epinephrin store of the adrenal constituted a full load, a good indication that protection of the gland from depletion during the experiment was relatively complete.

*Protocol.* Cat 444; male; weight, 2.9 kgm. Right adrenal excised and left denervated 8 days, left superior cervical ganglion excised 14 days previously. Under ether cut vago-sympathetics, excised stellate ganglia, prepared central end of right sciatic for stimulation.

		<i>Rate</i>	<i>Pressure</i>
10:40 a.m.	Before sciatic stimulation (8 cm.).....	133	132
	2 seconds after beginning stimulation.....	130	128
	30 seconds after beginning stimulation.....	132	130
10:45 a.m.	Before sciatic stimulation (5 cm.).....	133	112
	6 seconds after beginning stimulation.....	140	
	40 seconds after beginning stimulation.....	152	92
	60 seconds after beginning stimulation.....	145	106
10:50 a.m.	Before sciatic stimulation (8 cm.).....	127	108
	3 seconds after beginning stimulation.....	132	
	30 seconds after beginning stimulation.....	136	100
	45 seconds after beginning stimulation.....	131	104
10:55 a.m.	Before sciatic stimulation (6 cm.).....	121	120
	5 seconds after beginning stimulation.....	130	126
11:10 a.m.	Excised left adrenal (extraperitoneally)		
11:15 a.m.	Before sciatic stimulation (8 cm.).....	130	103
	3 seconds after beginning stimulation.....	133	96
	23 seconds after beginning stimulation.....	140	
	60 seconds after beginning stimulation.....	144	114
11:48 a.m.	Opened abdomen, tied coeliac axis and superior mesenteric artery		
11:50 a.m.	Before sciatic stimulation (6 cm.).....	139	74
	8 seconds after beginning stimulation.....	144	74
	3 seconds after stopping stimulation.....	142	72
	40 seconds after stopping stimulation.....	134	70

11:58 a.m.	Before sciatic stimulation (4 cm.).....	123	72
	2 seconds after beginning stimulation.....	130	77
	15 seconds after beginning stimulation.....	133	80
	40 seconds after beginning stimulation.....	137	81
	60 seconds after beginning stimulation.....	138	78
12:05 p.m.	Before sciatic stimulation (8 cm.).....	130	76
	2 seconds after beginning stimulation.....	133	80
	30 seconds after beginning stimulation.....	135	82

In cat 444 the greatest acceleration caused by sciatic stimulation before excision of the remaining adrenal was 19 beats. With repeated stimulations the acceleration effect declined. The last stimulation before excision gave only 9 beats. A weaker stimulation just after excision gave a maximum acceleration of 14 beats, and another stronger stimulation gave a maximum acceleration of 15 beats. Even after the abdomen was opened and the superior mesenteric artery and coeliac axis tied, a maximum acceleration of 15 beats was obtained in the absence of the adrenals.

In cat 441, also, while the greatest acceleration before excision of the remaining adrenal (17 beats) was not reached afterwards, the acceleration immediately after excision was the same as that obtained just before it (9 beats), although the stimulation in the latter case was the stronger.

*Protocol.* Cat 441; female; weight, 1.66 kgm. Right adrenal excised and left denervated 7 days previously. Under ether cut vago-sympathetics, excised stellate ganglia, prepared central end of right sciatic for stimulation.

		Rate	Pressure
10:55 a.m.	Before sciatic stimulation (10 cm.).....	219	100
	6 seconds after beginning stimulation.....	229	116
	20 seconds after beginning stimulation.....	228	
10:58 a.m.	Before sciatic stimulation (8 cm.).....	217	115
	6 seconds after beginning stimulation.....	223	122
11:02 a.m.	Before sciatic stimulation (6 cm.).....	208	106
	10 seconds after beginning stimulation.....	215	118
	30 seconds after beginning stimulation.....	213	
11:06 a.m.	Before sciatic stimulation (6 cm.).....	211	109
	5 seconds after beginning stimulation.....	217	
	18 seconds after beginning stimulation.....	228	120
	28 seconds after beginning stimulation.....	227	
11:10 a.m.	Before sciatic stimulation (10 cm.).....	207	113
	5 seconds after beginning stimulation.....	215	116
11:15 a.m.	Before sciatic stimulation (8 cm.).....	206	109
	5 seconds after beginning stimulation.....	222	139

		<i>Rate</i>	<i>Pressure</i>
11:20 a.m.	Before sciatic stimulation (7 cm.).....	216	116
	5 seconds after beginning stimulation.....	224	148
11:25 a.m.	Before sciatic stimulation (6 cm.).....	212	98
	5 seconds after beginning stimulation.....	221	119
11:40 a.m.	Excised left adrenal (lumbar route)		
11:42 a.m.	Before sciatic stimulation (8 cm.).....	214	86
	5 seconds after beginning stimulation.....	220	
	20 seconds after beginning stimulation.....	223	114
11:45 a.m.	Before sciatic stimulation (10 cm.).....	211	84
	5 seconds after beginning stimulation.....	214	100
	23 seconds after beginning stimulation.....	215	
11:51 a.m.	Before sciatic stimulation (9 cm.).....	210	70
	6 seconds after beginning stimulation.....	214	88
11:55 a.m.	Before sciatic stimulation (7 cm.).....	208	70
	6 seconds after beginning stimulation.....	210	84
11:58 a.m.	Before sciatic stimulation (8 cm.).....	211	68
	16 seconds after beginning stimulation.....	218	97
12:00 m.	Prepared central end of left sciatic for stimulation		
12:05 p.m.	Before stimulation of left sciatic (9 cm.).....	214	68
	30 seconds after beginning stimulation.....	217	72
12:10 p.m.	Before stimulation of right sciatic (10 cm.).....	215	68
	5 seconds after beginning stimulation.....	216	76
12:15 p.m.	Before stimulation of right sciatic (8 cm.).....	236	55
	5 seconds after beginning stimulation.....	242	62
	11 seconds after beginning stimulation.....	245	

The left adrenal weighed 0.226 gm. and contained 0.23 mgm. of epinephrin at end of experiment.

In the last cat (450) of this series to be mentioned a longer period (34 days) was allowed to elapse between the primary operation and the experiment, in order that the animal might have more fully recovered.

*Protocol.* Cat 450; male; weight, 2.96 kgm. Right adrenal excised and left denervated (with section of left splanchnic as usual) 34 days previously. Under urethane (5 gm.) cut vago-sympathetics; excised stellate ganglia; prepared central end of left sciatic for stimulation.

		<i>Rate</i>	<i>Pressure</i>
11:10 a.m.	Before sciatic stimulation (10 cm.).....	173	102
	During first 18 seconds of stimulation.....	178	114
	During next 20 seconds of stimulation.....	182	114
	Just after end of stimulation.....	185	
	30 seconds after end of stimulation.....	180	
11:14 a.m.	Before sciatic stimulation (9 cm.).....	174	100
	During first 12 seconds of stimulation.....	178	
	During next 16 seconds of stimulation.....	183	133
	Just after end of stimulation.....	191	126
	20 seconds after end of stimulation.....	189	129



		<i>Rate</i>	<i>Pressure</i>
11:18 a.m.	Before sciatic stimulation (8 cm.).....	171	97
	During first 16 seconds of stimulation.....	178	
	During next 17 seconds of stimulation.....	187	134
	Just after end of stimulation.....	192	134
	20 seconds after end of stimulation.....	190	
11:40 a.m.	Excised left adrenal (extraperitoneally)		
11:43 a.m.	Before sciatic stimulation (9 cm.).....	174	94
	During first 15 seconds of stimulation.....	179	130
	During next 20 seconds of stimulation.....	189	127
11:48 a.m.	Before sciatic stimulation (8 cm.).....	174	103
	During first 14 seconds of stimulation.....	178	132
	During next 20 seconds of stimulation.....	191	144
	Just after end of stimulation.....	200	150
	15 seconds after end of stimulation.....	198	128
	35 seconds after end of stimulation.....	194	127
11:58 a.m.	Before sciatic stimulation (7 cm.).....	177	96
	During first 17 seconds of stimulation.....	181	128
	During next 20 seconds of stimulation.....	192	138
	Just after end of stimulation.....	200	122
	20 seconds after end of stimulation.....	195	120
12:10 p.m.	Prepared peripheral end of right splanchnic (in thorax)		
12:12 p.m.	Before splanchnic stimulation (10 cm.).....	167	70
	During first 20 seconds of stimulation.....	176	96
	During next 20 seconds of stimulation.....	184	104
	Just after end of stimulation.....	186	92
12:15 p.m.	Before splanchnic stimulation (8 cm.).....	177	61
	During first 20 seconds of stimulation.....	175	70
	During next 20 seconds of stimulation.....	185	90
	Just after end of stimulation.....	189	86
12:21 p.m.	Before splanchnic stimulation (7 cm.).....	178	60
	During first 16 seconds of stimulation.....	179	93
	During next 20 seconds of stimulation.....	186	108
	Just after end of stimulation.....	187	96
12:26 p.m.	Before sciatic stimulation (7 cm.).....	179	62
	During first 20 seconds of stimulation.....	182	83
	During next 20 seconds of stimulation.....	180	72
12:35 p.m.	Cut left splanchnic in thorax		
12:36 p.m.	Before sciatic stimulation (7 cm.).....	181	63
	During first 18 seconds of stimulation.....	182	82
	During next 20 seconds of stimulation.....	181	78
	Just after end of stimulation.....	184	74
1:01 p.m.	Before splanchnic stimulation (7 cm.).....	183	46
	During first 16 seconds of stimulation.....	184	78
	During next 20 seconds of stimulation.....	196	88
	Just after end of stimulation.....	208	74
	15 seconds after end of stimulation.....	209	65
	22 seconds after end of stimulation.....	212	60

It will be seen from the protocol that a good acceleration was obtained on sciatic stimulation before removal of the remaining (already denervated) adrenal, (12 beats, 17 beats, 21 beats per minute in successive observations with different strength of stimulation). After removal of the adrenal the accelerations obtained were fully as great as before (15 beats, 26 beats, 23 beats per minute). When, however, in the absence of the adrenals, the right splanchnic was cut in the thorax sciatic stimulation caused practically no acceleration, and the rise of pressure was, of course, much reduced (fig. 5). This was not due to any change in the condition of the animal which rendered direct stimulation of the splanchnic less effective, for the rise of pressure and the acceleration on stimulating the peripheral end of the right splanchnic

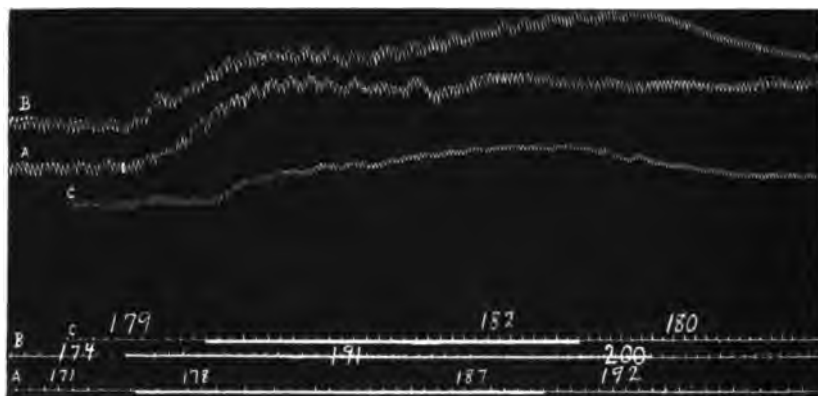


Fig. 5. Blood pressure curves from cat 450. A, sciatic stimulation before and B, after excision of remaining (already denervated) adrenal; C, after section of remaining splanchnic. Reduced to three-fifths.

was quite as great as before the last sciatic stimulation (as much as 29 beats per minute), (fig. 6). The failure of the heart reaction with sciatic stimulation after section of the right splanchnic (the left had been divided at the primary operation) is precisely what Cannon describes as occurring after removal of the adrenals. But in this animal the removal of the remaining adrenal did not affect the heart reaction, while subsequent section of the remaining splanchnic abolished it. This is incompatible with Cannon's interpretation of the failure of the reaction after section of the splanchnics as due entirely to interference with the epinephrin output. In the present experiment, to be logical, he would have to attribute the result to the loss of something coming from the liver (sugar?) or from the intestines, mobilized reflexly through the splanchnic.

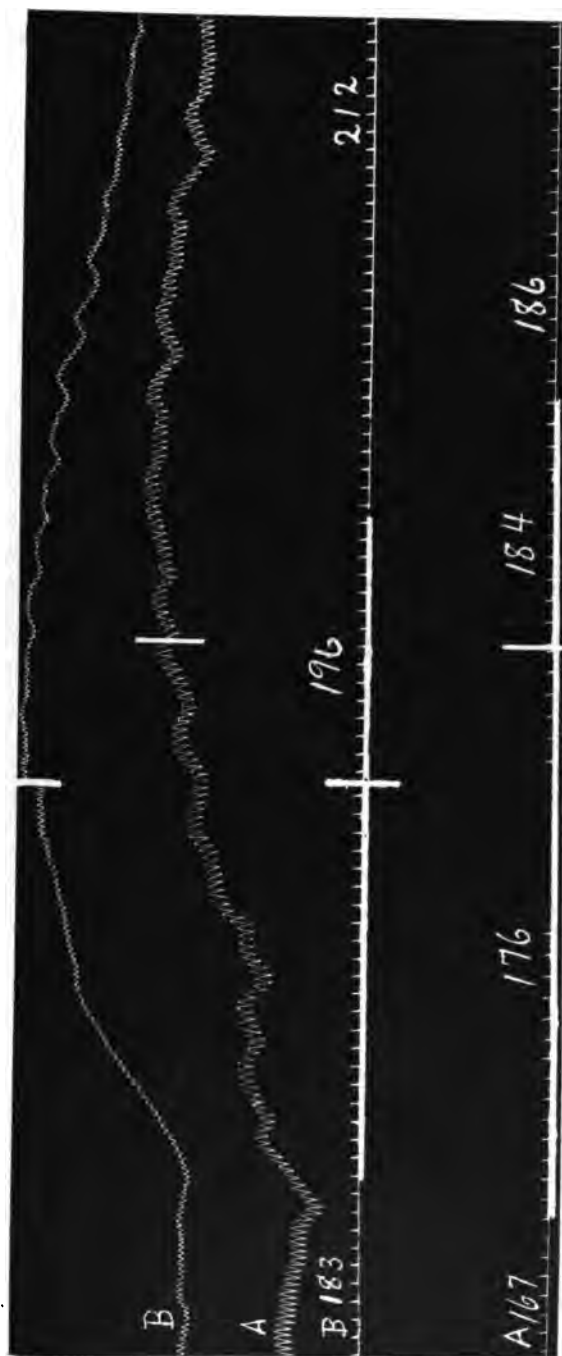


Fig. 6. Blood pressure curves from cat 450. A, splanchnic stimulation after excision of remaining (already denervated) adrenal; B, same, with stronger stimulus, 50 minutes later (7 seconds of each trace cut out to save space).

# EXPERIMENTS WITH AN INTERVAL BETWEEN REMOVAL OF THE TWO ADRENALS

To avoid division of one splanchnic and to make the first operation less severe than the ordinary operation for suppression of the epinephrin output, while making the second operation less severe than the removal of both adrenals at one time, a number of experiments were performed in which one adrenal was removed, and then, after an interval of 3 to 13 days, the observations on sciatic stimulation with denervation of the heart and excision of the remaining adrenal were made. In cat 440, for example, the experiment was performed 6 days after the removal of the right adrenal.

*Protocol.* Cat 440; male; weight, 2.31 kgm. Right adrenal excised 6 days previously. Under ether cut vago-sympathetics, excised stellate ganglia, prepared central end of right sciatic for stimulation.

		<i>Rate</i>	<i>Pressure</i>
10:45 a.m.	Before sciatic stimulation (10 cm.).....	226	134
	5 seconds after beginning stimulation.....	256	168
10:49 a.m.	Before sciatic stimulation (8 cm.).....	220	128
	6 seconds after beginning stimulation.....	253	171
	15 seconds after end of stimulation.....	264	156
11:00 a.m.	Excised left adrenal (extraperitoneally)		
11:02 a.m.	Before sciatic stimulation (8 cm.).....	212	124
	5 seconds after beginning stimulation.....	242	
	15 seconds after beginning stimulation.....	255	156
	23 seconds after beginning stimulation.....	250	156
	37 seconds after beginning stimulation.....	228	138
	60 seconds after beginning stimulation.....	218	134
11:05 a.m.	Before sciatic stimulation (6 cm.).....	213	119
	6 seconds after beginning stimulation.....	252	154
	28 seconds after beginning stimulation.....	247	
11:09 a.m.	Before sciatic stimulation (4 cm.).....	212	118
	5 seconds after beginning stimulation.....	230	133
	22 seconds after beginning stimulation.....	248	153
11:20 a.m.	Before sciatic stimulation (8 cm.).....	221	112
	6 seconds after beginning stimulation.....	236	130
	22 seconds after beginning stimulation.....	247	140
11:25 a.m.	Before sciatic stimulation (6 cm.).....	230	118
	8 seconds after beginning stimulation.....	241	130
11:28 a.m.	Before sciatic stimulation (4 cm.).....	225	133
	7 seconds after beginning stimulation.....	231	146
	43 seconds after beginning stimulation.....	227	130
11:45 a.m.	Exposed cord in midcervical region		
11:50 a.m.	.....	208	90

		Rate	Pressure
11:55 a.m.	Before total asphyxia (for 45 seconds).....	207	84
	3 seconds after beginning asphyxia.....	204	
	10 seconds after beginning asphyxia.....	225	88
	55 seconds after beginning asphyxia.....	210	88
12:05 a.m.	3 minutes after transection of cord between 4th and 5th cervical segments.....	193	51

As will be seen from the protocol, excellent heart reactions were obtained both before and after the removal of the remaining adrenal in cat 440. It is to be particularly noted that excision of the left adrenal left the blood pressure and heart rate practically unchanged.

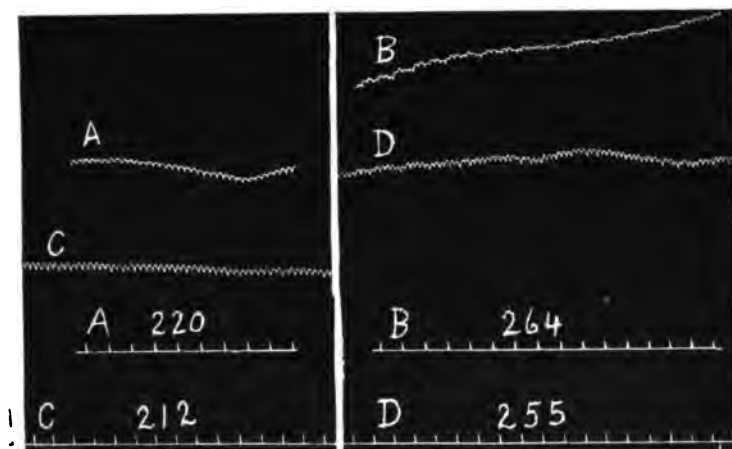


Fig. 7. Parts of blood pressure tracings from cat 440. A, before and B, a portion commencing 15 seconds after beginning of sciatic stimulation, before excision of remaining adrenal; C, before and D, 15 seconds after beginning of sciatic stimulation, after excision of remaining adrenal. Zero line moved up 41 mm.

The maximum acceleration for the last sciatic stimulation prior to removal of the left adrenal was 33 beats per minute, and for the first sciatic stimulation after removal of the adrenal 42 beats, the maximum pulse rate reached being practically identical in the two cases (253, 250). Samples of the curves used in counting the pulse rate are given in figure 7. The greater portion of each of the two curves, much reduced, is reproduced in figure 8, to show that excision of the second adrenal has not in any way essentially changed the vascular reaction. Here was a cat, then, without adrenals in which the acceleration produced by stimulation of the sciatic was actually greater than that

produced by a similar stimulation while one adrenal was still intact. Yet, according to Cannon, the last acceleration, like the first, must have been due solely to augmented epinephrin output from the adrenals. It should be noted that the exposure of the cord in the midcervical region led to a drop of blood pressure to 90 mm. of mercury and a corresponding slowing of the heart to 208 beats a minute. Subsequent transection of the cord caused the blood pressure to fall to 51 mm. of mercury and the heart rate to 193. It must be remembered that the adrenals were out, and the decrease in the heart rate can have nothing to do with absence of adrenal epinephrin. Cannon has emphasized the fact that after removal of the adrenals the pulse rate drops. Our observations show that this is always true provided the blood pressure

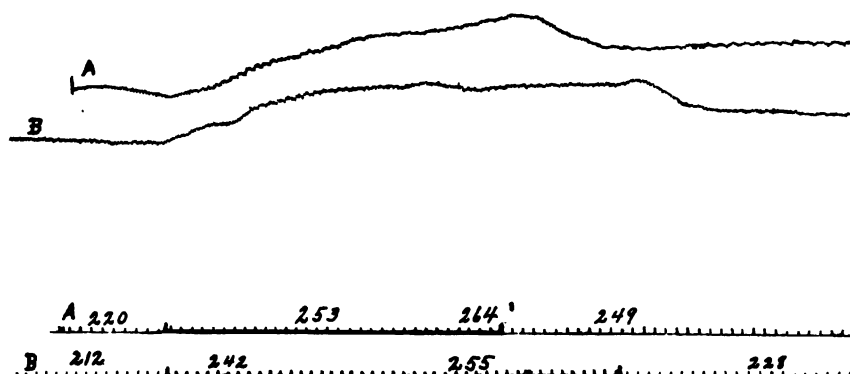


Fig. 8. Blood pressure curves from cat 440. A, sciatic stimulation before and B, after removal of remaining adrenal. Reduced to one-half.

falls decidedly. But if the pressure is maintained there is not necessarily any sensible slowing of the heart. The diminution in the pulse rate is, therefore, no index of the previous rate of output of epinephrin. This statement of observed facts is made without prejudice to the question whether the epinephrin liberated at the ordinary rate, under our experimental conditions, is capable of exerting an influence upon the heart, including its rate. We have brought forward some evidence that there is such an influence. But it may not be obvious after suppression of the epinephrin output when the blood pressure is well maintained. In cat 440 repeated stimulation of the sciatic caused some exhaustion of the heart reaction after removal of the last adrenal. But this, of course, is true of all reflexes, and in the case of the heart reaction can occur also with intact adrenals.

In the next experiment (cat 443) an example is given in which the heart acceleration elicited by sciatic stimulation was small from the beginning, but quite as good reactions were obtained after removal of the second adrenal as before (greatest acceleration before removal of the adrenal, 7 beats in one observation and 9 beats in another; after removal, 10 beats in one observation and 11 beats in another). It will be noted that as the blood pressure continued to fall progressively the heart rate diminished also, and this was not related to the adrenalectomy. Thus half an hour after removal of the adrenal, the pulse rate was 160 and the blood pressure 76. When the blood pressure had fallen to 50 the heart rate was 147. Before the removal of the adrenal the pulse rate was 180, 179 and 200 in 3 observations. After the adrenalectomy it was 182, the blood pressure being 83 mm. of mercury instead of 110 mm. at the last observation. As in other protocols counts of the heart rate at different parts of the curve and the results of stimulation of different strengths are given in order to show that the maximum accelerations quoted were really the maximum obtainable, under our conditions, in each animal.

*Protocol.* Cat 443; female; weight, 1.53 kgm. Right adrenal excised 7 days, left superior cervical ganglion excised 13 days previously. Under ether cut vago-sympathetics, excised stellate ganglia, prepared central end of left sciatic for stimulation.

		Rate	Pressure
10:55 a.m.	Before sciatic stimulation (10 cm.) <sup>7</sup> .....	180	116
	6 seconds after beginning stimulation.....	187	148
10:59 a.m.	Before sciatic stimulation (9 cm.).....	179	106
	6 seconds after beginning stimulation.....	183	128
11:04 a.m.	Before sciatic stimulation (9 cm.).....	200	110
	20 seconds after beginning stimulation.....	209	122
	45 seconds after beginning stimulation.....	205	116
11:15 a.m.	Excised left adrenal (extraperitoneally).....		
11:16 a.m.	Before sciatic stimulation (10 cm.).....	182	83
	5 seconds after beginning stimulation.....	180	85
11:20 a.m.	Prepared central end of right sciatic.....		
11:27 a.m.	Before stimulation of left sciatic (8 cm.).....	165	80
	23 seconds after beginning stimulation.....	168	100
11:43 a.m.	Before stimulation of right sciatic (6 cm.).....	161	75
	2 seconds after beginning stimulation.....	160	
	6 seconds after beginning stimulation.....	164	
	17 seconds after beginning stimulation.....	170	94
	3 seconds after end of stimulation.....	171	

<sup>7</sup> The eye reactions were the same as described in footnote to protocol of cat 190, p. 319.

		<i>Rate</i>	<i>Pressure</i>
11:46 a.m.	Before sciatic stimulation (6 cm.).....	160	76
	10 seconds after beginning stimulation.....	169	98
	6 seconds after end of stimulation.....	170	
11:50 a.m.	Before stimulation of left sciatic (6 cm.).....	155	62
	6 seconds after beginning stimulation.....	157	70
12:07 p.m.	Before stimulation of left sciatic (6 cm.).....	147	50
	7 seconds after beginning stimulation.....	151	
	30 seconds after beginning stimulation.....	153	56

In both of the experiments hitherto cited in this section (cats 440 and 443) the blood pressure after removal of the second adrenal remained good, practically unchanged in the first cat and only moderately lowered in the other. In the next experiment (cat 438) a considerable fall of pressure accompanied the operation for removal of the second adrenal. There is no reason to attribute this to the fact that the operation was done by the abdominal route. A similar fall of pressure occurred in cat 439 after removal of the second adrenal extraperitoneally by the lumbar route.

*Protocol.* Cat 438; female, weight 1.91 kgm. Right adrenal excised 3 days previously. Under urethane (3 gm.) cut vago-sympathetics, excised stellate ganglia, prepared central end of left sciatic for stimulation.

		<i>Rate</i>	<i>Pressure</i>
12:08 p.m.	Before sciatic stimulation (6 cm. to 4 cm.).....	189	122
	After increasing strength of stimulus (5 cm.).....	194	
	After increasing strength of stimulus (4 cm.).....	200	
	At end of stimulation.....	213	154
12:30 p.m.	Before sciatic stimulation (6-4 cm.).....	206	110
	4 seconds after beginning stimulation.....	220	140
	After increasing strength of stimulus (4 cm.).....	237	178
12:45 p.m.	Opened abdomen, excised left adrenal.....		
12:50 p.m.	Before sciatic stimulation (4 cm.).....	130	40
	During first 15 seconds stimulation.....	138	
	After increasing strength of stimulus.....	143	52
	After further increasing strength of stimulus.....	150	64
1:03 p.m.	Before sciatic stimulation (6-4 cm.).....	161	
	5 seconds after beginning stimulation.....	170	
1:10 p.m.	Before sciatic stimulation (6-4 cm.).....	161	66
	5 seconds after beginning stimulation.....	162	
	30 seconds after beginning stimulation.....	166	83
1:15 p.m.	Before sciatic stimulation (4 cm.).....	171	60
	10 seconds after beginning stimulation.....	170	74
1:20 p.m.	Before total asphyxia for 1 minute.....	175	64
	Counting from end of asphyxia.....	225	64



In cat 438 it should be noted that with the fall of pressure from well over 100 mm. to 40 mm. of mercury, associated with the adrenalectomy, the pulse rate diminished from 206 before the previous sciatic stimulation to 130. To attribute this diminution of 76 beats per minute entirely to the lack of the epinephrin from the second adrenal is, we believe, unwarranted. As the pressure gradually rose later on in the experiment, be it remembered in the absence of the adrenals, the pulse rate increased also to 161, 171 and 175 with pressures of 60 to 66. In spite of the rather low blood pressure which, however, showed a tendency to improve, a fair acceleration of the heart was elicited by stimulation of the sciatic after removal of the second adrenal (as much as 20 beats per minute, compared with 24 beats and 31 beats in two observations before the adrenalectomy).

*Protocol.* Cat 439; male; weight, 1.93 kgm. Right adrenal excised 5 days previously. Under urethane (3 gm.) cut vago-sympathetics, excised stellate ganglia and prepared central end of left sciatic for stimulation.

		Rate	Pressure
11:30 a.m.	Before sciatic stimulation (10 cm.).....	210	125
	4 seconds after beginning stimulation.....	217	146
	After increasing strength of stimulus (8 cm.).....	234	160
11:40 a.m.	Before sciatic stimulation (6 cm.).....	200	100
	3 seconds after beginning stimulation.....	222	142
11:50 a.m.	Excised left adrenal (extraperitoneally).....		
11:53 a.m.	Before sciatic stimulation (6 cm.).....	178	54
	15 seconds after beginning stimulation.....	181	68
	After increasing strength of stimulus (5 cm.).....	180	66
12:00 m.	Before sciatic stimulation (6 cm.).....	179	56
	6 seconds after beginning stimulation.....	183	72
12:10 p.m.	Prepared central end of right sciatic.....		
12:12 p.m.	Before stimulation of right sciatic (7 cm.).....	180	40
	5 seconds after beginning stimulation.....	183	58
	After increasing strength of stimulus (3 cm.).....	183	49
12:20 p.m.	Before sciatic stimulation (6 cm.).....	177	40
	5 seconds after beginning stimulation.....	182	44
	After end of stimulation.....	182	40
12:25 p.m.	Before intravenous injection of Ringer.....	173	20
	Immediately after injection of 100 cc. Ringer.....	188	46
	30 seconds after end of injection.....	190	68

In cat 439 although the blood pressure, immediately after removal of the second adrenal, was a little higher than in cat 438, it tended to grow progressively worse, sinking at last to 20 mm. of mercury, whereas the opposite tendency was seen in cat 438. Only very trifling accelerations of the heart were caused by sciatic stimulation after the adre-

nalectomy, although before fair reactions were obtained (as much as 24 beats in one observation and 22 beats per minute in another). The changes of blood pressure produced by stimulation of the sciatic were also small. Since the heart reaction depends upon a reflex or reflexes, as already pointed out, it necessarily fails or is diminished when the reflex arcs have deteriorated under the influence of a low blood pressure and a poor blood flow. Toward the end of the experiment an injection of Ringer's solution raised the blood pressure from 20 to 68 mm. and the pulse rate from 173 to 190. Epinephrin, of course, could have nothing to do with this acceleration. That the relative failure of the heart reaction on sciatic stimulation after removal of the second adrenal was not dependent upon the impossibility of a reflex increase in the epinephrin output but upon other, probably circulatory conditions, is indicated in the next experiment (cat 446), the last to be cited in this section.

*Protocol.* Cat 446; female; weight, 1.67 kgm. Right adrenal excised 6 days previously. Under urethane cut vago-sympathetics, excised stellate ganglia, prepared central end of right sciatic for stimulation.

		<i>Rate</i>	<i>Pressure</i>
11:00 a.m.	Before sciatic stimulation (8 cm.).....	185	115
	10 seconds after beginning stimulation.....	200	152
	20 seconds after beginning stimulation.....	203	
	30 seconds after beginning stimulation.....	204	123
11:02 a.m.	Before sciatic stimulation (9 cm.).....	185	104
	10 seconds after beginning stimulation.....	200	130
	32 seconds after beginning stimulation.....	199	113
11:20 a.m.	Excised left adrenal (extraperitoneally).....		
11:22 a.m.	Before sciatic stimulation (9 cm.).....	148	57
	10 seconds after beginning stimulation.....	150	64
	34 seconds after beginning stimulation.....	150	61
11:30 a.m.	Intravenous injection of 100 cc. Ringer.....		
11:50 a.m.	Before sciatic stimulation (7 cm.).....	164	80
	10 seconds after beginning stimulation.....	174	
	25 seconds after beginning stimulation.....	178	115
	40 seconds after beginning stimulation.....	173	
11:55 a.m.	Before sciatic stimulation (8 cm.).....	161	76
	4 seconds after beginning stimulation.....	163	
	22 seconds after beginning stimulation.....	168	97
	40 seconds after beginning stimulation.....	162	82
12:03 p.m.	Before sciatic stimulation (6 cm.).....	162	72
	5 seconds after beginning stimulation.....	170	88
	23 seconds after beginning stimulation.....	171	
	42 seconds after beginning stimulation.....	169	70
12:06 p.m.	Opened abdomen, tied renal arteries and veins.....		

		<i>Rate</i>	<i>Pressure</i>
12:11 p.m.	Clipped abdominal aorta and stimulated sciatic;		
	before stimulation (7 cm.).....	171	74
	10 seconds after beginning stimulation.....	173	
	35 seconds after beginning stimulation.....	176	83
	After removal of clip.....	172	53
12:20 p.m.	Intravenous injection of 50 cc. Ringer.....		
12:25 p.m.	Clipped abdominal aorta and stimulated sciatic;		
	before stimulation (7 cm.).....	170	90
	10 seconds after beginning stimulation.....	179	
	28 seconds after beginning stimulation.....	176	96
	After release of aorta.....	174	81
12:30 p.m.	Prepared central end of left sciatic for stimulation....		
12:32 p.m.	Clipped abdominal aorta and stimulated left sciatic;		
	before stimulation (7 cm.).....	173	88
	10 seconds after beginning stimulation.....	180	
	30 seconds after beginning stimulation.....	185	110
	45 seconds after beginning stimulation.....	180	88
	10 seconds after release of aorta.....	174	63
12:35 p.m.	Clipped abdominal aorta and stimulated left sciatic;		
	before stimulation (6 cm.).....	163	70
	10 seconds after beginning stimulation.....	169	78
	31 seconds after beginning stimulation.....	171	60
	10 seconds after release of aorta.....	166	45

After removal of the second adrenal the blood pressure fell from over 100 mm. of mercury to 57 mm. and the pulse rate dropped from 185 to 147 beats per minute. Sciatic stimulation caused practically no acceleration of the heart (3 beats per minute as compared with 15 beats before the adrenalectomy) and very little rise of blood pressure (7 mm.). The pressure went on falling and Ringer's solution was injected, which brought the blood pressure up to 80 mm. Stimulation of the sciatic nerve now caused an acceleration of 14 beats per minute and a rise of blood pressure of 35 mm. of mercury. Another stimulation caused an acceleration of 12 beats when the blood pressure had been raised to 88 mm. of mercury by temporary clipping of the abdominal aorta, the clip being put on and the pressure allowed to become constant, which only required a fraction of a minute, before the beginning of stimulation. Samples of portions of the tracings used for counting the pulse rate are given in figures 9 and 10, and the whole curves (much reduced) from which these portions were taken, in figure 11.

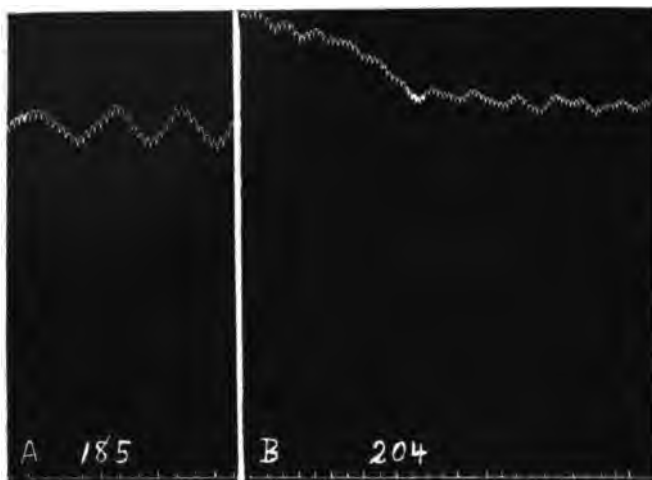


Fig. 9. Parts of blood pressure tracings from cat 446. *A*, before and *B*, a portion commencing 19 seconds after beginning of sciatic stimulation. Reduced to four-fifths.

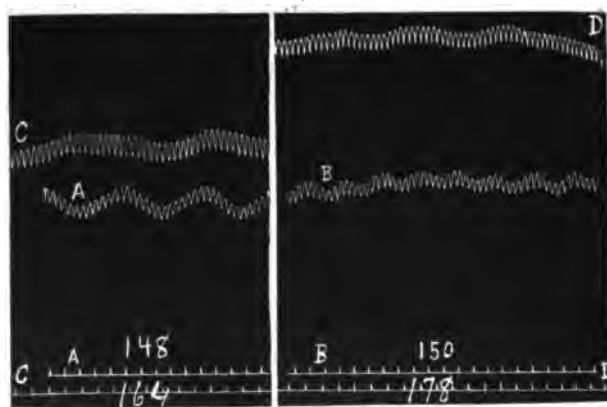


Fig. 10. Parts of blood pressure tracings from cat 446. *A*, before and *B*, a portion commencing 16 seconds after beginning of sciatic stimulation, after excision of remaining adrenal; *C*, before and *D*, 23 seconds after beginning of sciatic stimulation, after intravenous injection of Ringer. Reduced to four-fifths.

## EXPERIMENTS IN WHICH BOTH ADRENALS WERE REMOVED AT ONE TIME

It seems probable that in most of the cats with one adrenal removed some time before the experiment the interval was too short for the full advantage of this procedure to be obtained, if there is an advantage. The results were, nevertheless, decisive as regards the question at issue. To check the matter a series of observations was made in which both adrenals were removed, as carefully as possible, at the time of the experiment on the denervated heart reaction. The protocol of cat 449 is cited as an example of experiments in which the adrenals were extirpated after opening the abdomen.

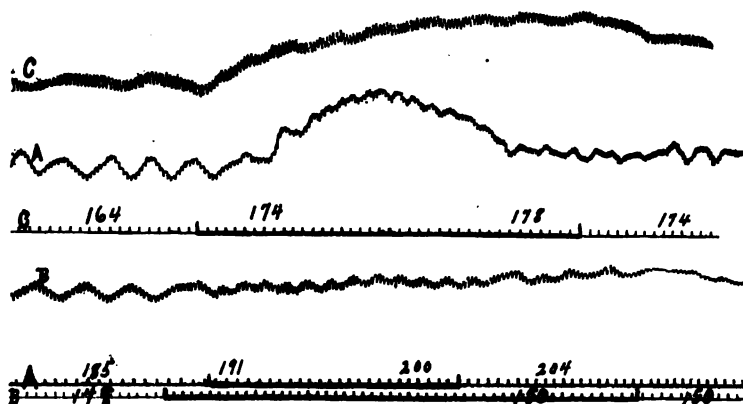


Fig. 11. Blood pressure curves from cat 446. A, sciatic stimulation before and B, after excision of remaining adrenal; C, after intravenous injection of Ringer. Reduced to one-half.

*Protocol.* Cat 449; male; weight, 3.38 kgm. Under urethane (6 gm.) cut vago-sympathetics, excised stellate ganglia, prepared central end of left sciatic for stimulation.

		Rate	Pressure
12:00 m.	Before sciatic stimulation (8 cm.)	285	125
	10 seconds after beginning stimulation	293	170
	25 seconds after beginning stimulation	294	
12:12 p.m.	Before sciatic stimulation (6 cm.)	256	110
	15 seconds after beginning stimulation	280	147
	40 seconds after beginning stimulation	283	130
12:35 p.m.	Opened abdomen, excised both adrenals		
12:36 p.m.	Before sciatic stimulation (6 cm.)	228	70
	12 seconds after beginning stimulation	238	124
	35 seconds after beginning stimulation	238	90

		<i>Rate</i>	<i>Pressure</i>
12:50 p.m.	Before sciatic stimulation (5 cm.).....	223	74
	15 seconds after beginning stimulation.....	241	127
	30 seconds after beginning stimulation.....	238	89
1:05 p.m.	Before sciatic stimulation (7 cm.).....	222	73
	10 seconds after beginning stimulation.....	237	130
	30 seconds after beginning stimulation.....	234	92
1:15 p.m.	Prepared peripheral end of left splanchnic in thorax for stimulation.		
1:20 p.m.	Before splanchnic stimulation (9 cm.).....	226	58
	10 seconds after beginning stimulation.....	231	111
	30 seconds after beginning stimulation.....	223	69
1:25 p.m.	Before splanchnic stimulation (7 cm.).....	221	59
	8 seconds after beginning stimulation.....	229	110
1:40 p.m.	Before sciatic stimulation (6 cm.).....	221	52
	10 seconds after beginning stimulation.....	231	92
	26 seconds after beginning stimulation.....	230	
1:45 p.m.	Before sciatic stimulation (6 cm.).....	223	52
	12 seconds after beginning stimulation.....	232	98
	40 seconds after beginning stimulation.....	233	64

Two sciatic stimulations were made before extirpation of the adrenals. The first yielded a maximum acceleration of 9 beats only, but the initial heart rate was unusually great. In the second stimulation the maximum acceleration was 24 to 27 beats per minute, the initial rate being decidedly lower than in the first observation. After removal of the adrenals, accelerations of 10, 18 and 15 beats were obtained in 3 successive sciatic stimulations, and even at the end of the experiment, when the blood pressure had fallen considerably, an acceleration of 10 beats was gotten. It is scarcely necessary to point out that it would be futile to try to determine from such figures whether epinephrin was taking any sensible share in the reaction before the adrenalectomy, and if so, how much. For the maximum acceleration of which a given heart is capable at different stages in an experiment, under the influence of the changes produced by stimulation of the sciatic other than any possible change in the epinephrin output, must vary with the condition of the heart, and this with the blood flow on which its nutrition depends. Stimulation of the peripheral end of a splanchnic nerve after removal of both adrenals caused also distinct acceleration, as much as 8 to 10 beats per minute, in this experiment although, of course, some further fall of blood pressure had been caused by division of the nerve. Portions of the curves used for counting the heart rate in the last sciatic stimulation prior to removal of the adrenals and the second stimulation after removal are given in figure 12, and a reduction

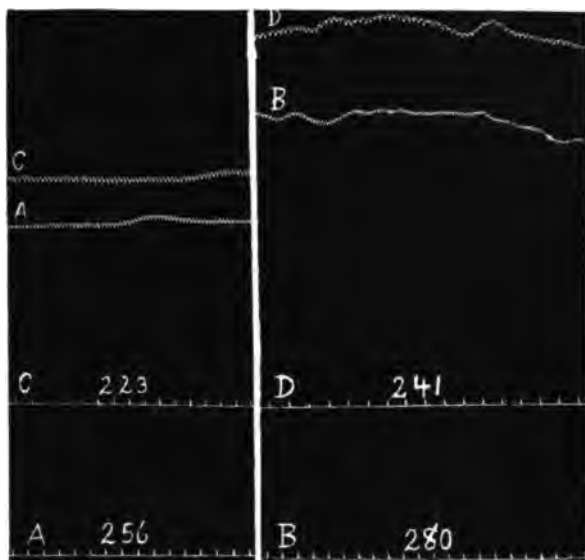


Fig. 12. Parts of blood pressure tracings from cat 449. *A*, before and *B*, a portion commencing 15 seconds after beginning of sciatic stimulation, before excision of both adrenals; *C*, before and *D*, 15 seconds after beginning of sciatic stimulation, after excision of both adrenals. Reduced to four-fifths.

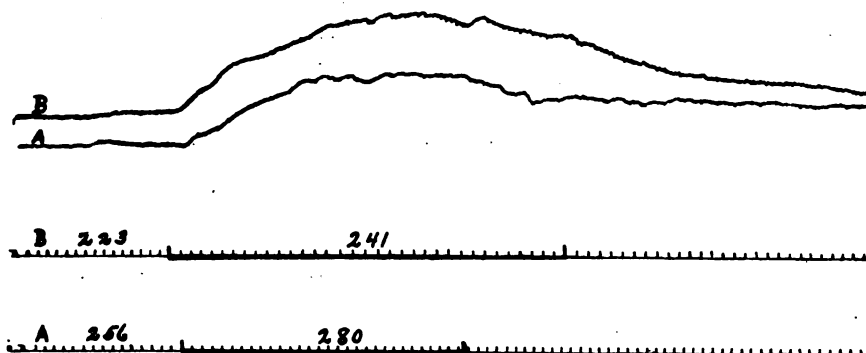


Fig. 13. Blood pressure curves from cat 449. *A*, sciatic stimulation before and *B*, after excision of both adrenals. Reduced to one-half.

of the greater part of the two curves in figure 13. It will be seen that neither the blood pressure reaction nor the acceleration was essentially modified by the absence of the adrenals. The blood pressure, although decidedly lower after the adrenalectomy, was still good (70 to 80 mm. of mercury, as compared with 110 mm. before the operation).

In the next experiment (cat 448) the adrenals were removed extra-peritoneally by the lumbar route. The results were practically the same as in cat 449, in which the abdomen had been opened.

*Protocol.* Cat 448; male; weight, 3.29 kgm. Under urethane (6 gm. in two doses) cut vago-sympathetics, excised stellate ganglia, prepared central end of left sciatic for stimulation.

		Rate	Pressure
11:43 a.m.	Before sciatic stimulation (10 cm.).....	217	132
	10 seconds after beginning stimulation.....	226	158
	30 seconds after beginning stimulation.....	232	144
11:45 a.m.	Before sciatic stimulation (8 cm.).....	210	135
	10 seconds after beginning stimulation.....	234	170
	30 seconds after beginning stimulation.....	242	149
12:20 p.m.	Extirpated both adrenals (extraperitoneally).....		
12:25 p.m.	Before sciatic stimulation (8 cm.).....	165	90
	6 seconds after beginning stimulation.....	171	103
	25 seconds after beginning stimulation.....	170	
12:32 p.m.	Before sciatic stimulation (7 cm.).....	164	87
	10 seconds after beginning stimulation.....	181	132
	26 seconds after beginning stimulation.....	184	128
	35 seconds after beginning stimulation.....	182	118
12:40 p.m.	Sciatic now stimulated twice with an interval of only 2 minutes, to fatigue the reaction, and then at .....		
12:50 p.m.	Before sciatic stimulation (6 cm.).....	173	92
	10 seconds after beginning stimulation.....	181	126
	35 seconds after beginning stimulation.....	183	
1:03 p.m.	Sciatic again stimulated twice in rapid succession and then at .....		
1:14 p.m.	Before sciatic stimulation (6 cm.).....	171	76
	10 seconds after beginning stimulation.....	175	107
	40 seconds after beginning stimulation.....	177	
1:20 p.m.	Opened abdomen, tied renal arteries and veins.....		
1:24 p.m.	Before sciatic stimulation (6 cm.).....	174	88
	4 seconds after beginning stimulation.....	181	110
	30 seconds after beginning stimulation.....	181	98
1:33 p.m.	Stimulated sciatic with abdominal aorta clipped. Before clipping aorta.....	170	77
	Before sciatic stimulation.....	177	100
	10 seconds after beginning stimulation.....	184	
	30 seconds after beginning stimulation.....	186	114
	After release of aorta.....	184	
	30 seconds after release of aorta.....	185	86



1:37 p.m.	Stimulated sciatic with abdominal aorta and vena cava clipped;		
	Before stimulation (7 cm.).....	Rate 174	Pressure 76
	8 seconds after beginning stimulation.....	180	
	30 seconds after beginning stimulation.....	182	100
	10 seconds after removal of clips from aorta and cava.....	176	68
2:21 p.m.	Before sciatic stimulation (7 cm.).....	159	47
	10 seconds after beginning stimulation.....	159	60
	Just after end of stimulation.....	158	
2:25 p.m.	Before sciatic stimulation (4 cm.).....	155	43
	10 seconds after beginning stimulation.....	161	55
	40 seconds after beginning stimulation.....	161	

Accelerations as great as 20 beats per minute were obtained after removal of the adrenals. Before removal the maximum acceleration seen in two observations with different strengths of stimulus was 15 and 32 beats respectively. The blood pressure fell from 135 to 90 mm. of mercury after the adrenalectomy. When the sciatic was then repeatedly stimulated with only short intervals between the successive stimulations, the heart reaction, as estimated by the maximum acceleration, diminished but about the same absolute rate (180 to 183) was reached at the height of the acceleration. Later on in the experiment, when the abdomen had been opened and the renal vessels tied, an acceleration of 7 beats per minute was caused by clipping the abdominal aorta just above the bifurcation, as is done in the collection of blood from the cava pocket. This acceleration was accompanied by a rise of blood pressure from 77 to 100 mm. of mercury. When the sciatic was now stimulated with the aorta still clipped (the clip was put on only a short time before stimulation of the sciatic so that the excitation of the nerve should not be interfered with) a further acceleration of 9 beats a minute occurred. Sciatic stimulation gave a similar acceleration with both abdominal aorta and cava clipped, as in making the pocket. At the end of the experiment, when the blood pressure had fallen to 40 to 50 mm. of mercury, no sensible acceleration, or with stronger stimulation only a small one, could be elicited through the sciatic and the rise of blood pressure was also small. But it would surely be absurd to attribute this belated failure of a reaction which had been well obtained after the adrenalectomy to the absence of a reflexly excited outpouring of epinephrin.

As to varying the strength of stimulation, it should be stated that we did not consider there would be any point in merely comparing the reaction obtained before and after the removal of the adrenals with

practically the same nominal strength of stimulus, since the excitability of the reflex mechanisms cannot be counted upon to remain the same, especially where considerable changes of blood pressure have occurred. What we tried to do in all the experiments was to elicit as large a reaction as possible, both before and after elimination of the adrenals, using for this purpose the strength of stimulation which seemed most effective. The strongest stimuli were not generally the best. Not infrequently we found that stimuli of the same strength as gave the maximum acceleration before removal of the adrenals did so after their removal also. But sometimes it was necessary to increase the stim-

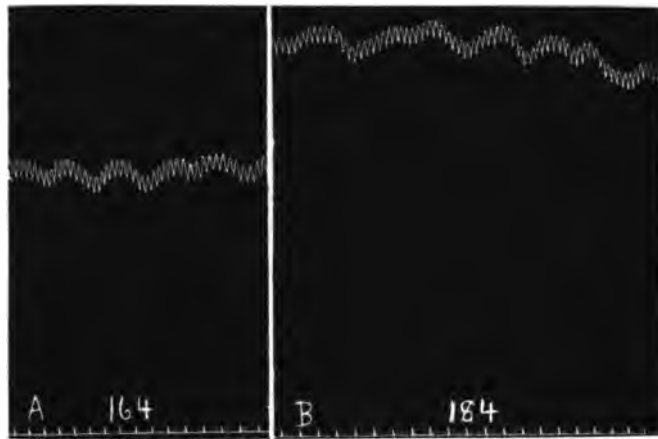


Fig. 14. Parts of blood pressure tracings from cat 448. *A*, before and *B*, a portion commencing 26 seconds after beginning of sciatic stimulation, after excision of both adrenals. Reduced to four-fifths.

ulus. This is mentioned because if the same strength of stimulus is employed before and after removal of the adrenals, a small, or even no acceleration might be obtained after the operation, which would not mean that the reaction had disappeared because of the loss of the adrenals, but that the excitability of the mechanisms concerned in it had diminished. The same is, of course, true of the vasomotor reflex, which we took as an indicator of effective stimulation.

Portions of a curve from cat 448, showing acceleration on sciatic stimulation after removal of the adrenals, are reproduced in figure 14, and the greater part of the curve on a reduced scale in figure 15.

*Protocol.* Cat 437; female; weight, 1.61 kgm. Left superior cervical ganglion excised one month previously. Under urethane (3 gm.) cut vago-sympathetics, excised stellate ganglia, prepared central end of left sciatic for stimulation.

		Rate	Pressure
11:46 a.m.	Before sciatic stimulation <sup>*</sup> .....	260	116
	5 seconds after beginning stimulation.....	274	150
11:50 a.m.	Opened abdomen, tied off renal arteries and veins.....		
11:55 a.m.	Before sciatic stimulation.....	253	60
	10 seconds after beginning stimulation.....	264	84
12:00 m.	Before stimulation of sciatic with abdominal aorta clipped.....	250	64
	2 seconds after beginning stimulation.....	270	88
	15 seconds after beginning stimulation.....	273	
12:05 p.m.	Before stimulation of sciatic with abdominal aorta and cava clipped.....	255	60
	4 seconds after beginning stimulation.....	278	84
12:10 p.m.	Excised both adrenals.....		
12:11 p.m.	Before sciatic stimulation.....	203	44
	4 seconds after beginning stimulation.....	232	59
2:15 p.m.	Before sciatic stimulation.....	214	42
	10 seconds after beginning stimulation.....	225	53

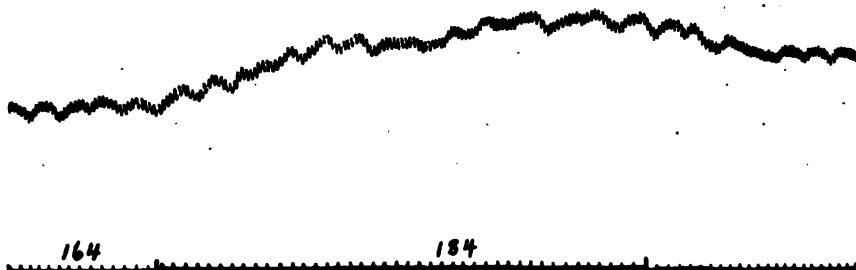


Fig. 15. Blood pressure curve from cat 448. Sciatic stimulation after excision of both adrenals. Reduced to one-half.

In cat 437, in which a superior cervical ganglion had been excised a month previously and in which, therefore, eye reactions were also available, the adrenalectomy was performed at a different stage of the experiment, and after the abdomen had been opened for some time and observations made on the results of sciatic stimulation with the abdominal aorta and cava clipped, as in the collection of blood from the adrenals. The demonstration that the heart reaction is obtained after removal of the adrenals was, if anything, more striking than in many of the other experiments. Sciatic stimulation yielded a maximum

<sup>\*</sup> The eye reactions were those described in the footnote to protocol of cat 190, p. 319.

acceleration of 14 beats per minute before the abdomen was opened. After opening the abdomen and tying off the renal vessels, the blood pressure had fallen to 60 mm. of mercury as compared with 116 mm. at the beginning of the experiment. An acceleration of 11 beats was given by stimulation of the sciatic. The abdominal aorta was now clipped and a short interval allowed for any effect on blood pressure and heart rate to develop, and then the sciatic was stimulated. The heart rate compared with that immediately before stimulation was increased by 23 beats per minute. An equal acceleration was given in another observation, in which the abdominal aorta and inferior cava (above the junction of the iliac veins) were clipped in the same way shortly before stimulation. In such observations the clips were not removed till the portion of the curve to be used for counting the heart beats had been completed. Both adrenals were then excised and thereafter an acceleration of 29 beats was caused by stimulation of the sciatic. It is surely impossible to reconcile the results of such an experiment with Cannon's statements: *a*, that after opening the abdomen it is very rare to obtain any acceleration of the heart by sciatic stimulation; *b*, that preparing the cava pocket for collection of adrenal blood, as practised by Biedl, Hoskins and McClure (14), ourselves and other investigators, renders it impossible to detect the great increase in epinephrin output which according to him is indicated by the heart reaction; and *c*, that removal of the adrenals abolishes the reaction because it is due entirely to a reflexly excited increase in the secretion of epinephrin.

The last experiment to which we shall allude (cat 447) was one in which sciatic stimulation caused only a small acceleration at the beginning of the experiment, accompanied by insignificant changes of blood pressure. The cat was only half grown, but it is not known whether this had anything to do with the relatively small effects. The reaction was not essentially modified by removal of the adrenals, or subsequently by clipping the abdominal aorta. The blood pressure was diminished from about 100 mm. to little over 50 mm. of mercury after the adrenalectomy, and the heart rate was also diminished from over 200 to 175 beats a minute. As the blood pressure fell still lower toward the end of the experiment, the heart rate diminished further to 160 beats a minute.

*Protocol.* Cat 447; female; weight, 1.24 kgm. Under ether cut vago-sympathetics; excised stellate ganglia; prepared central end of left sciatic for stimulation.

		Rate	Pressure
11:00 a.m.	Before sciatic stimulation (8 cm.).....	203	115
	Just after end of stimulation.....	218	110
11:02 a.m.	Before sciatic stimulation (10 cm.).....	210	100
	12 seconds after beginning stimulation.....	207	100
11:05 a.m.	Before sciatic stimulation (9 cm.).....	205	110
	10 seconds after beginning stimulation.....	209	112
11:11 a.m.	Before sciatic stimulation (7 cm.).....	206	110
	12 seconds after beginning stimulation.....	209	118
	20 seconds after beginning stimulation.....	212	
11:18 a.m.	Before sciatic stimulation (9 cm.).....	202	104
	15 seconds after beginning stimulation.....	208	108
11:40 a.m.	Opened abdomen, excised both adrenals		
11:43 a.m.	Before sciatic stimulation (7 cm.).....	175	53
	15 seconds after beginning stimulation.....	181	63
	40 seconds after beginning stimulation.....	184	
11:46 a.m.	Before sciatic stimulation (9 cm.).....	179	55
	15 seconds after beginning stimulation.....	182	55
	30 seconds after beginning stimulation.....	183	55
11:48 a.m.	Before sciatic stimulation (5 cm.).....	172	54
	Just after beginning stimulation.....	177	
	10 seconds after beginning stimulation.....	172	54
11:53 a.m.	Before stimulation of sciatic with abdominal aorta clipped (8 cm.).....	172	55
	6 seconds after beginning stimulation.....	171	59
	After end of stimulation.....	179	
12:10 p.m.	Before stimulation of sciatic with abdominal aorta clipped (7 cm.).....	160	49
	6 seconds after beginning stimulation.....	160	51
	25 seconds after beginning stimulation.....	166	
12:15 p.m.	Before sciatic stimulation (8 cm.).....	163	35
	10 seconds after beginning stimulation.....	166	48
	30 seconds after beginning stimulation.....	164	

*The slowing of the denervated heart after interference with the epinephrin output.* It is not easy to demonstrate conclusively that elimination of the epinephrin output of the adrenals causes a slowing of the denervated heart, indicating that the ordinary output, under the conditions of our experiments, is capable of exerting an influence upon the heart. For when the elimination of the epinephrin output is brought about by removal of the adrenals in an acute experiment, the result may be complicated by a slowing associated with a fall of blood pressure. Nevertheless, as stated previously (15), "evidence of a relation of the

normal epinephrin output to the heart rate seems to be afforded by a comparison of the effects produced in cats on the rate by excision of the stellate ganglia after previous section of the vagi when the adrenal epinephrin is normally entering the circulation and in the absence of epinephrin." This is illustrated in table 1.

Although the number of animals is small, the results are suggestive. In the three cats which had been subjected to the adrenal operation mentioned there was a decided diminution in the heart rate after removal of the ganglia. In cat 235 the rate was unusually slow

TABLE 1

CAT	BEFORE		AFTER REMOVAL OF STELLATES		REMARKS
	Rate	Pressure	Rate	Pressure	
231	216	176	(a)152	104	33 days after adrenal operation
			(b)164	82	(b) 10 minutes later than (a)
233	193	87	168	76	36 days after adrenal operation
235	142	106	(a)144	90	34 days after adrenal operation
			(b)129	78	(b) 2 minutes later than (a)
234	266	152	255	122	
236	250	184	(a)249	168	
			(b)233	118	(b) 7 minutes later than (a)
237	192	126	(a)187	110	
			(b)178	104	(b) 4 minutes later than (a)
193	172	120	188	114	Stellates removed an hour after beginning of experiment after repeated splanchnic stimulation
195	181	78	145	52	

In the first three cats the right adrenal had been removed and the nerves of the left cut. The last 5 cats were normal animals. All were anesthetized with urethane.

(142 beats per minute) before the excision of the stellate ganglion and immediately after excision of the second ganglion it was 144. But it at once began to fall and in 2 minutes was 129. An acceleration is not infrequently seen during the operation for removal of the ganglia, due it may be supposed to reflex stimulation of the accelerantes or direct mechanical stimulation or possibly to indirect effects produced through vasomotor changes. The full effect of the elimination of the accelerator nerves may then not be seen for a minute or two.

In cat 231 the epinephrin output as estimated on adrenal vein blood by assay on rabbit intestine segments could not have been one-twenty-

fifth of the normal average. In cat 233 it could not have been more than one-twentieth of the normal average (intestine and pupil assay), and in cat 235 it could not have been one-three-hundredth of the normal; even the adrenal blood sample with the slowest flow (0.6 gm. per minute) was shown by the intestine segment test to have a smaller concentration than 1:300,000,000 adrenalin. In the five normal cats in table 1 only in one was a decided slowing of the heart observed after excision of the stellate ganglia and this was associated with a marked fall of blood pressure.

TABLE 2

CAT	BEFORE		AFTER REMOVAL OF REMAINING ADRENAL		Days after operation
	Rate	Pressure	Rate	Pressure	
441	219	100	214	86	7
444	131	141	137	138	8
450	173	102	174	94	34
438	195	104	(a)195*	74	3
439	210	125	(b)130	40	
440	226	134	178	54	5
443	180	116	212	124	6
445	161	128	182	83	7
446	185	115	143	86	5
			147	57	6
			164	80†	

In the first three cats the right adrenal had been excised and the nerves of the left cut. In cat 445 the left and in the others the right adrenal had been removed. Cats 440, 441, 443, 444, 445 were etherized, the others were under urethane.

\* (a) immediately and (b) 4 to 5 minutes after removal of adrenal. The blood pressure was falling continuously during this time.

† After injection of Ringer's solution.

In table 2 are shown the heart rates and blood pressures before and after removal of the remaining adrenal from three cats in which the right adrenal had been excised and the nerves of the left cut, and in seven cats from which one adrenal had been removed at a previous operation. The vago-sympathetics had been cut and the stellate ganglia excised at the beginning of the experiment. In the three cats whose remaining adrenal had been denervated no diminution in the pulse rate was caused by excision of the gland. As already remarked, the fact that the blood pressure was practically unaltered by removal

of the previously denervated adrenal in these cats makes it difficult to estimate the influence of the suppression of the epinephrin output from the gland upon the result. In four out of the other seven cats a substantial slowing of the heart was found after removal of the remaining adrenal. In another of the seven cats (438) although the rate was unchanged immediately after removal of the remaining adrenal the blood pressure was steadily falling and 5 minutes later the pulse rate was only 130.

In table 3 are given the heart rates and blood pressures in five normal cats before and after removal of both adrenals at one operation, the vago-sympathetics having been cut and the stellate ganglia excised at the beginning of the experiment. In every case there was a diminu-

TABLE 3

CAT	BEFORE		AFTER REMOVAL OF BOTH ADRENALS		ANESTHETIC
	Rate	Pressure	Rate	Pressure	
447	203	115	175	53	Ether
448	218	132	165	90	Ether
449	285	125	228	70	Urethane
437	256	116	203	44	Urethane
195*	146	44	131	38	Urethane

\* In cat 195 the adrenals were removed about an hour after removal of stellates. The rate just before removal of the adrenals was 145.

tion in the heart rate, but this again was in every case associated with a fall of blood pressure.

Table 4 shows the heart rates and blood pressures in fourteen normal cats after section of the vago-sympathetics and excision of the stellate ganglia. In three dogs anesthetized with morphine and ether the pulse rates after denervation of the heart were 106, 153 and 173, beats per minute, with blood pressures of 92, 72 and 38 mm. of mercury respectively. That different anesthetics may affect the pulse rate of the denervated heart differently may be assumed, e.g., chloroform diminishes the rate in dogs according to v. Anrep (1). So far as can be judged from a relatively small number of observations in our own experiments, the difference between ether and urethane was not conspicuous.



If all the results on the cats are brought together, some further suggestive points seem to emerge. Thus, in twelve out of twenty<sup>9</sup> normal cats with both adrenals intact the pulse rate was over 200 a minute after denervation of the heart. In two out of seven cats with one adrenal previously removed and in only two out of nine cats with one adrenal previously removed and the nerves of the other cut, was the pulse rate over 200 after denervation of the heart. The average pulse rate of the twenty cats with both adrenals intact, after denervation of the heart, was 209 beats per minute; the average for the nine cats whose epinephrin output had been previously interfered with by excision of one adrenal and section of the nerves of the other 170 beats

TABLE 4

CAT	RATE	PRESSURE	CAT	RATE	PRESSURE
201	177	54	190	250	123
202	145	92	191	240	124
215	219	134	196	212	72
175	188		198	233	60
	176	82*	199	137	113
176	160		200	210	84
177	185	132	436	203	56
179	169	80			

\* 2 hours after the first observation.

In cats 201, 202 and 215 the right adrenal had been excised and the nerves of the left cut 18 days, 8 days and 59 days respectively before the operation, the others were normal cats. All were anesthetized with urethane, cat 436 more deeply than the rest.

per minute; and the average for the seven cats from which one adrenal had been previously removed 184 beats per minute. A possible influence of the previous operation as such, apart from interference with epinephrin output, where the interval was only a few days is not excluded, but is not discernible in the tables.

<sup>9</sup> In 4 additional normal cats the heart rates and blood pressures after denervation of the heart, but before adrenalectomy, were 266 (214), 284 (145), 265 (145), and 150 (66). In the last cat the abdomen had been opened before the heart was denervated. After removal or ligation of both adrenals the corresponding numbers were 222 (114), 283 (102), 255 (130), and 143 (57). In a fifth cat the rate after denervation of the heart was 248 and the blood pressure 150 mm. of mercury.

## DISCUSSION AND SUMMARY

It has been shown by us that the acceleration of the heart caused by stimulation of the central end of the sciatic is in no way a reaction by which the rate of output of epinephrin from the adrenals can be estimated, or changes in that rate demonstrated, as claimed by Cannon. This is proved by the following facts:

a. Clipping of the adrenal veins has no demonstrable influence upon the occurrence and magnitude of the heart reaction caused by sciatic stimulation, although it markedly diminishes or abolishes reactions which are known to be genuine reactions for epinephrin, such as the dilatation of the pupil following stimulation of the peripheral end of a splanchnic nerve.

b. Acceleration of the denervated heart on sciatic stimulation is well obtained in cats which have been allowed to survive after removal of one adrenal and section of the nerves of the other, an operation which is known to abolish or greatly diminish the epinephrin output. In such cats the reaction is still elicited after the remaining adrenal has been removed.

c. Good acceleration of the heart can be elicited by stimulation of the sciatic in cats from which both adrenals have been removed, either in two operations with an interval for recovery interposed, or at one operation.

d. When the reaction disappears after removal of the adrenals this is not because of the absence of increased epinephrin discharge on stimulation of the sciatic but for other reasons, such as deterioration in the condition of the animal (fall of blood pressure, etc.) which interferes with the reflex or reflexes necessarily involved in the reaction or with the capacity of the heart to markedly accelerate its beat.

e. Contrary to Cannon's statement, the reaction is well obtained after opening the abdomen. It can be elicited after ligation of the renal vessels, abdominal aorta and inferior cava, as practised in forming a cava pocket for collection of adrenal vein blood. If the reaction indicates a greatly increased output of epinephrin reflexly induced by stimulation of the sciatic, as assumed by Cannon, we could not have failed to detect the increase by the direct method of collecting adrenal vein blood and assaying its epinephrin content on rabbit segments. But our results were negative (16).

As regards the real mechanism of the acceleration of the denervated heart caused by sciatic stimulation, we desire to point out, once for

all, that the onus of explaining this probably complex indirect reaction, which Cannon erroneously interprets as indicating increased epinephrin secretion, does not rest upon us at all. It is for Doctor Cannon to exclude, if he can, by control experiments, other possible factors in the reaction which he attributes solely to epinephrin. Our position is simply this. We have investigated the influence of stimulation of the sciatic and brachial nerves upon the rate of epinephrin output by a direct method, correct in principle and free from ambiguity, and have obtained negative results. Doctor Cannon states that by means of an indirect method (the denervated heart reaction) he obtains a positive result. We show that this reaction cannot yield any information as to the rate of epinephrin output from the adrenals or as to changes in that rate, since it is obtainable when the epinephrin output of the adrenals is abolished. And here we are entitled to rest our case, not, of course, claiming that sensory stimulation *cannot* increase the epinephrin output, but that no increase has hitherto been proved.

However, certain fairly obvious suggestions may be made as to factors which may play a part in the heart reaction under discussion, a reaction probably made up of more than one component. One is the larger amount of epinephrin sent through the coronary circulation and perhaps the greater concentration of it, owing to the vasomotor changes produced by stimulation of the sciatic (11), (15). It may be pointed out that even if Cannon's statement that the reaction cannot be obtained in the absence of the adrenals had been found correct, that of itself would only have shown that epinephrin is essentially concerned. For it might be due to the redistribution of the epinephrin without any increase in the rate of output.

Cannon attempts to invalidate this suggestion by an experiment in which he prevents a rise of pressure in the carotid during sciatic stimulation by compression of the chest, and yet obtains an acceleration of the heart. Now this is a quite complex experiment, and Doctor Cannon is doing several other things which may affect the heart besides keeping the pressure in the aorta constant. One of the things he is doing is impeding the venous return. The epinephrin, even if its rate of output remains unchanged, must, therefore, be diluted with a smaller proportion of indifferent blood in the right heart. Blood with a greater concentration of epinephrin must accordingly be passing through the coronaries, and as the concentration will increase in the same measure as the slowing in the venous return necessary to prevent rise of pressure, approximately the same amount of epinephrin will pass through the coronaries per unit of time as with a similar sciatic stimulation without compression of the chest. If then the ordinary output of epinephrin was a factor in the acceleration without compression it may be expected to exert the same influence during compression. In other words, an increase in the amount of epinephrin passing per unit of time through the coronary circulation during sciatic stimulation is not prevented by compressing the chest, so as to keep the blood pressure in the aorta from rising,

even if no increase in the rate of output of epinephrin has occurred, and the experiment is without significance for the question at issue. That no further acceleration occurred when the blood pressure rose after releasing the chest, is also just as intelligible, so far as epinephrin is a factor, on the assumption that the epinephrin output was not increased as on the assumption that it was increased by stimulation of the sciatic. For if the epinephrin concentration remained unchanged and the coronary blood flow was increased on decompressing the chest, an increased amount of epinephrin would pass through the coronaries per minute whether the output had been augmented by stimulation of the sciatic or not. The fact is, however, that with the increase in the venous inflow to the heart the concentration of epinephrin in the blood of the right heart must be proportionally diminished. But why, in any case, should a further increase in the heart rate have been expected, since the acceleration was already 36 beats a minute, the same as without compression of the chest? As there is no possibility that epinephrin is the sole factor in the heart reaction, as elicited reflexly, the point need not be labored. Whatever other factors are ordinarily concerned in the reaction, apart from the rise of arterial blood pressure, may be expected to act as well during compression of the chest as before. The possibility that a special factor, the gross interference with the mechanism of the heart, especially the filling and pressure of the right side and with the respiration might exert an influence in this experiment is not excluded.

We ourselves made two experiments (cats 198, 199) in which the rise of pressure on sciatic stimulation was largely prevented by hemorrhage, controlled by a mercury valve. The animals were anesthetized with urethane. In cat 198 before sciatic stimulation the pulse was 225, the blood pressure 78; during stimulation the pulse rate rose to 253 and the blood pressure to 192 mm. of mercury. In the next observation the pulse was 233 and the blood pressure 60 before stimulation. During stimulation the blood pressure was prevented from rising beyond 90 mm. of mercury, and the pulse rate increased only to 242 beats per minute. Blood (mixed with salt solution) was reinjected. Before stimulation of the sciatic the pulse rate was 272, the pressure 96. During stimulation the pulse rate rose to 286 with a pressure of 168 mm. of mercury. The sciatic was now stimulated while the pressure was prevented, by hemorrhage, from changing (it rose from 88 to 92). The pulse rate before stimulation was 258 and during stimulation 259. This experiment might seem to show that the acceleration was largely prevented by keeping the blood pressure from rising. But in the other cat a different result was obtained. In cat 199 the following pulse rates and blood pressures, the latter in parentheses, were recorded. Before stimulation 137 (113), during stimulation 215 (196). Before stimulation 130 (108), during stimulation with hemorrhage 193 (116-90). Before stimulation 144 (100), during stimulation 206 (162). Before stimulation 145 (102), during stimulation with hemorrhage 194 (115). Only these two experiments were made. For on reflection it was seen that this method also could not lead to any definite conclusion. If the epinephrin were the only factor in the acceleration, its concentration in the blood coming to the heart would increase as the mass of the circulating blood diminished, even provided that no increase were taking place in the rate of output.

Besides the direct accelerating action of epinephrin upon the heart there is another way in which the normal output of epinephrin may possibly play a part

in the acceleration caused by stimulation of the sciatic, by sensitizing the heart to the action of other factors, such as a rise of blood pressure, which in the absence of epinephrin might not be so effective. In this connection we recall the observation of v. Anrep (1) on the influence of adrenalin upon the power of the heart to adapt itself by changes in its tone to changes in the arterial pressure.

Whatever share epinephrin may take in the acceleration reaction it cannot be the only factor and is probably not the most important one, since excellent heart reactions can be obtained in the absence of the adrenals, provided that good vascular reflexes, as evidenced by the change of blood pressure, are elicited. The most obvious of the changes caused by stimulation of the central end of the sciatic, the rise of blood pressure, is the one which seems to be most intimately related to the heart acceleration. Since this relation is seen after, as before, elimination of the adrenals, the most direct suggestion is that the better blood flow through the coronary circulation is an important factor in the acceleration, either by raising the nutritive condition and the excitability of the mechanism in which the beat originates or by acting upon a local accelerator mechanism. An action of the increased blood pressure as such is not excluded. Indeed, long ago Johansson (17) pointed out that the acceleration of the heart (after section of the vago-sympathetics and excision of the stellate ganglia) caused by stimulation of the peripheral end of a splanchnic nerve and of the cut cervical cord, was dependent on the abruptness of the rise of pressure. At the time of Johansson's work, nothing was known of the secretory innervation of the adrenals, and it might be asked whether the whole acceleration in his experiments was not due to increased epinephrin output. This question, in our opinion, must be answered in the negative. For stimulation of the cord with one splanchnic cut produced in general a much greater acceleration than stimulation of one splanchnic, without any obvious reason why it should have caused a greater liberation of epinephrin, and this greater acceleration was accompanied by a larger rise of blood pressure. Further the dogs were curarized and curara (18) depresses the conductivity of the efferent epinephrin secretory path. We do not, however, know how much importance should be attached to this, as our work with curara was done on cats and was concerned only with the spontaneous liberation of epinephrin.

It is significant that the accelerations observed by him, taken in relation to the blood pressure changes, are of the same order of magnitude as those obtained by us with splanchnic and sciatic stimulation, whether with the adrenal veins clipped or in the absence of the adrenals. From our own observations, recorded in the preceding pages, it cannot be doubted that a rise of pressure, caused by stimulation either of the sciatic or the peripheral end of the splanchnic nerve, is associated with an acceleration of the denervated heart, unrelated to any immediate action of epinephrin. The maximum acceleration, as in Johansson's observations, may not be reached till the blood pressure has again begun to decline.

We agree with Johansson and with Lehndorff (19) that the manner in which the rise of pressure is produced is important as regards its action upon the heart. The latter observer remarks that raising the pressure by compressing the aorta seldom causes an acceleration, and that when acceleration is caused it is only slight. He was never able by inducing an asphyxial rise of pressure to elicit

the characteristic changes in the heart's action which he found on splanchnic stimulation. Guthrie and Pike (20) also found compression of the aorta inefficient. We have seen a definite acceleration, e.g., in cat 190 (see protocol), clipping the aorta in the thorax increased the pulse rate from 249 to 260 beats a minute, but this was a much smaller acceleration than that caused by splanchnic stimulation just before, although the increase of arterial pressure was greater when the aorta was clipped. In other cases we have seen no acceleration when the aorta was clipped in the thorax. Clipping of the abdominal aorta has been sometimes seen to cause some acceleration with a moderate rise of pressure, in the absence of the adrenals. The character of the blood sent to the heart is, not the same when the thoracic as when the abdominal aorta at the bifurcation is clipped, the liver and other viscera not being interfered with in the latter case. Apart altogether from the possible effect of epinephrin when the splanchnic is stimulated, the mechanical conditions under which the heart works are very different when the aorta is clipped and when the central end of the sciatic or the peripheral end of the splanchnic is stimulated, particularly as regards the venous inflow.

Cannon seems to criticise us for quoting the experiments of Guthrie and Pike on the influence of increased pressure of the perfusion fluid in accelerating the excised heart, as if this represented the sum total of our knowledge. We quoted this work, which was done in the laboratory of one of us, in a footnote to another paper (11), to show, and we believe it does show, that under certain conditions the heart, deprived of extrinsic innervation, can respond to changes of blood pressure by very considerable changes in frequency. When we stated that there is nothing strange about an increase in the rate of the denervated heart *in situ* when the central end of the sciatic or the peripheral end of the splanchnic is stimulated, and went on to say that it is obviously dependent upon the better blood flow through the coronary vessels, we had before us much of the evidence given on preceding pages that marked acceleration could be produced by stimulation of both of these nerves, in the absence of any possible output of epinephrin from the adrenals, and that the acceleration was associated with a rise of arterial pressure. Of the results of other observers on the heart *in situ*, we had in mind particularly the elaborate paper of Johansson (17) already referred to. The experiments of Martin and of Knowlton and Starling on the heart-lung preparation, which Cannon cites, were familiar to us, but we saw no point in quoting in a footnote observations which threw no light upon the accelerations we were obtaining by stimulation of the sciatic, under conditions which eliminated the liberation of epinephrin from the adrenals. Of course, the vasomotor reactions expressed in the rise of blood pressure under discussion might possibly have other actions upon the denervated heart, in addition to their most obvious action, the increased blood flow through the coronaries. It has been pointed out by various writers that it is not the same thing, as regards the heart rate, whether changes are induced in the arterial or in the venous pressure.

In the case of sciatic stimulation it might still be argued that the rise of pressure is only a sign that the stimulus is effective for some other reflex or reflexes on which the acceleration depends essentially. This is theoretically true, but any reflex which affects the composition of the blood, as sciatic stimulation may do (by causing hyperpnoea, increased reflex muscular action, possibly increased

mobilization of sugar or other changes in the liver through the splanchnics), must affect the heart concomitantly with the vasomotor reflex which increases the blood flow through the coronaries. In any case, the experiments on stimulation of the splanchnic in the absence of epinephrin output indicate the rise of arterial pressure as the most obvious of the factors associated with the heart reaction studied.

In two cats (one normal, 447, and one in which the epinephrin output had been interfered with, 444, both etherized), we have seen an acceleration, when sciatic stimulation elicited no rise of pressure either before or after elimination of the adrenals. But then there was evidence of effective stimulation, not only in the increased respiratory movements and respiratory blood pressure waves but also in a small depression of the blood pressure, succeeded by some rise after stoppage of the stimulation. In such a case the question presents itself, whether it is certain that in every individual the heart is completely severed from the central nervous system by section of the vagi and excision of the stellate ganglia. We repeat that, having shown, as we believe, that Cannon's supposed proof, by means of the heart reaction, of augmented epinephrin output through stimulation of the sciatic is illusory, we do not consider that the explanation of the mechanism of the reaction is our concern. We have simply made some suggestions. Nor do we judge it necessary to discuss in this paper his supposed proof of the augmenting action of asphyxia and of emotional excitement upon the output. Clearly all his conclusions upon this matter stand or fall together. It may be mentioned, however, that we have observed acceleration of the heart, induced by asphyxia, after removal of the adrenals.

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## THE EFFECT OF ACIDS, ALKALIES AND SALTS ON CATALASE PRODUCTION

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The acids used were hydrochloric, propionic, acetic and butyric; the alkalies or alkaline salts, sodium carbonate, sodium phosphate, ammonium carbonate and sodium acetate; the neutral salts, disodium phosphate, ammonium chloride; and the acid salt, monosodium phosphate. The animals were rabbits and dogs. The method of administration and the amounts of the substances used will be given in the description of the experiments. The catalase of the blood was determined before as well as at intervals after introducing the materials into the alimentary tract of the animal. The determinations were made by adding 1 cc. of blood to hydrogen peroxide in a bottle and the amount of oxygen liberated in 10 minutes was taken as a measure of the catalase content of the cubic centimeter of blood. The dog's blood was used undiluted while the rabbit's blood was diluted 1 to 5 with 0.9 per cent sodium chloride. The effect on the catalase of the jugular blood of the introduction of the acids, alkalies and salts into the alimentary tract of rabbits and dogs is shown in figure 1. The figures along the ordinates represent percentage increase or decrease in catalase and those along the abscissae time in minutes.

It may be seen under *acids* that the introduction of 1.5 gram per kilo of acetic acid dissolved in 75 cc. of water per kilo into the stomach of rabbits increased the catalase of the blood 22 per cent in one rabbit and 12 per cent in another in 90 minutes; propionic acid increased the blood catalase 16 per cent; butyric, 10 per cent; that 1.5 gram per kilo of hydrochloric acid dissolved in 75 cc. of water per kilo decreased the blood catalase 7 per cent in one rabbit, 9 per cent in another rabbit; and that 3 grams per kilo of hydrochloric acid decreased the catalase 28 per cent. While these percentages are in most cases small, at the same time they represent large differences in the amount of oxygen liberated by the blood from hydrogen peroxide. An increase or decrease



of 20 per cent in catalase, for example, represents an increase or decrease of about 100 cc. of oxygen liberated.

It may be seen further in figure 1 under *carbonates*, *phosphates* and *sodium acetate* that the introduction of these substances into the upper part of the intestine of dogs produced an increase in the catalase of the blood of the jugular. The amounts of these salts used were 10 grams per kilo dissolved in 75 cc. of water per kilo, with the exception of

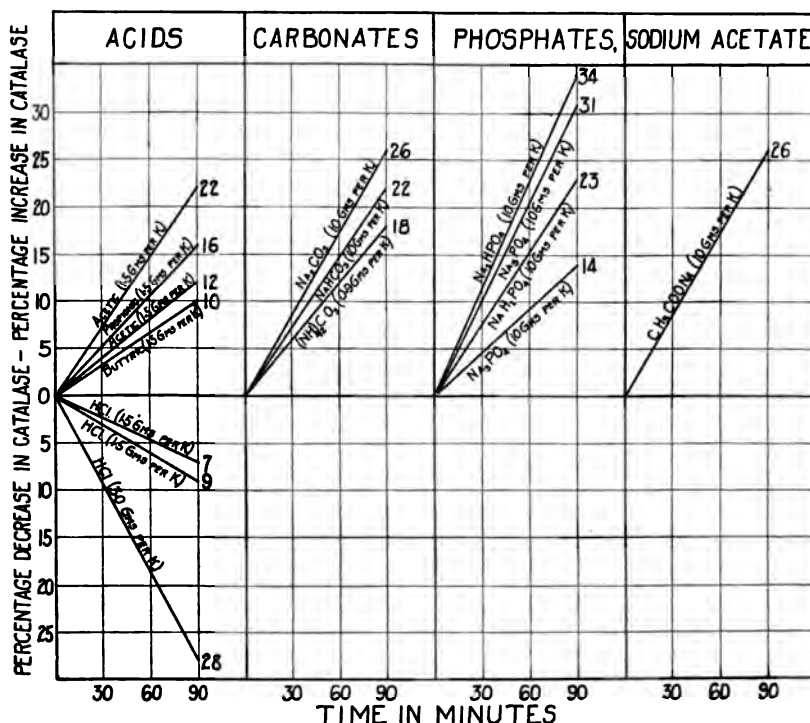


Fig. 1. Curves showing effect in vivo of the substances named in the chart on the catalase content of the jugular blood.

ammonium carbonate, 0.9 gram per kilo of this salt being used. After opening the abdominal wall of dogs while under ether, the materials were injected into the upper part of the small intestine. The wound was sewed up and the ether discontinued after the introduction of the substances.

The question that arises in this connection is, how is the increase as well as the decrease in catalase, observed in the preceding experi-

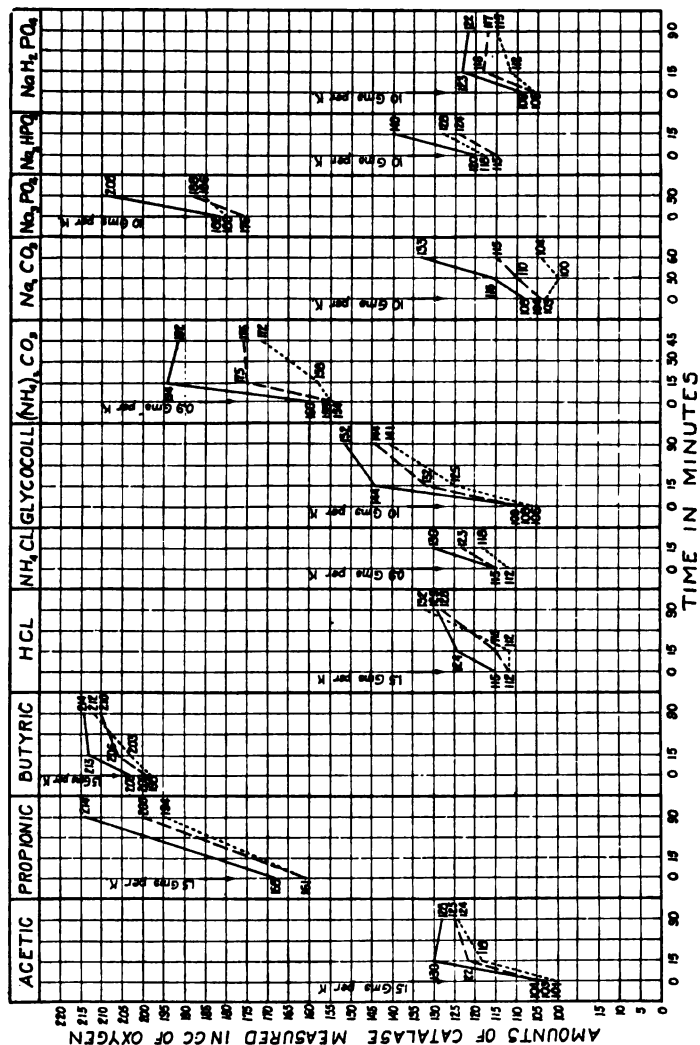


Fig. 2. Curves showing effect in vivo of the substances named in the chart on the catalase content of the blood. The continuous line curves show amount of catalase in the blood of the liver, the dash line curves the amount in the blood of the portal vein, and the dotted line curves the amount in the blood of the jugular vein.

ments, brought about. We (1) had already found that the ingestion of food, for example, produced an increase in catalase by stimulating the alimentary glands, particularly the liver, to an increased output of this enzyme, and that narcotics produced a decrease in catalase by decreasing its output from the liver and by direct destruction. So we naturally turned to the alimentary glands, particularly the liver, in looking for an explanation of the change in the amount of the blood catalase observed in the preceding experiments.

After opening the abdominal wall of a dog while under ether, catalase determinations were made using 1 cc. of blood from the liver, portal and jugular veins. The material to be studied was then introduced into the intestine and catalase determinations were made at fixed intervals. The results of the determinations are given in figure 2. The figures along the ordinate indicate amounts of oxygen liberated from hydrogen peroxide in 10 minutes by 1 cc. of blood, and the figures along the abscissae time in minutes. The continuous line curves were constructed from data obtained from the blood of the liver, the dash line curves from the blood of the portal, and the dotted line curves from the blood of the jugular vein.

Under *acetic* it may be seen that previous to the introduction into the intestine of a dog of 1.5 gram per kilo of acetic acid, dissolved in 75 cc. of water per kilo, 1 cc. of blood from the liver liberated 103 cc. of oxygen in 10 minutes from hydrogen peroxide; 1 cc. of blood from the portal liberated 104 cc. of oxygen, and 1 cc. from the jugular 101 cc. of oxygen. Fifteen minutes after the introduction of the acetic acid, the liver blood liberated 130 cc. of oxygen, the portal blood 122 cc. and the jugular blood 118 cc. Similarly, it may be seen that 90 minutes after the introduction of the acetic acid the liver blood liberated 128 cc. of oxygen, the portal blood 125 cc. and the jugular 124 cc.

By comparing these figures it may be seen that the liver blood, particularly during the first fifteen minutes after the introduction of the acetic acid, liberated more oxygen from hydrogen peroxide than did the portal blood, and that this blood in turn liberated more oxygen than did the jugular blood. This is taken to mean that the acetic acid was stimulating the alimentary glands, particularly the liver, to an increased output of catalase. It may be seen further that propionic, butyric and hydrochloric acids, ammonium chloride, glycocoll, ammonium carbonate, sodium carbonate and the phosphates produced an increase in catalase in a similar manner, namely, by stimulating the liver to an increased production of this enzyme.

The question that arises in this connection is, does the increase or decrease in the amount of oxygen liberated in the preceding experiments represent an actual increase or decrease in catalase, or does it simply represent a change in catalytic activity brought about by a decrease in the acidity of the hydrogen peroxide. So far as I have been able to find, Jacobson (2) was the first to show that catalytic activity is greatly decreased by acids. This work has been repeated and confirmed by several investigators (Loevenhart (3); Issajew (4); Senter (5); Winternitz and Rogers (6); Mendel and Leavonworth (7); Bodansky (8)) and extended to include the effect of acid salts, alkalies and alkaline salts on catalytic activity. The description of a simple experiment will suffice to show the effect of acids and alkalies on catalytic activity. If catalase determinations are made using equal quantities (1 cc.) of blood and neutral, slightly acid and slightly alkaline hydrogen peroxide, it will be found that the amount of oxygen liberated from the neutral and slightly alkaline hydrogen peroxide will be the same, while that liberated from the slightly acid hydrogen peroxide will be less. By increasing the acidity of the hydrogen peroxide, the amount of oxygen liberated will be decreased more and more until the action of the catalase is completely inhibited, and no oxygen is liberated. By the addition of an alkali (sodium hydroxide) to the acid hydrogen peroxide, the amount of oxygen liberated will be increased until a certain maximum is reached. Further addition of alkali at this point will begin to decrease the amount of oxygen liberated. It should be mentioned in this connection that alkalies are not nearly so effective in decreasing catalytic activity as the acids. Hence the amount of oxygen liberated from acid hydrogen peroxide can be increased by simply neutralizing the acid of the hydrogen peroxide by means of an alkali, or it can be decreased by making the hydrogen peroxide more acid.

Since in our experiments acids and alkalies were used, it might be thought by some that what we have been observing is not an increase or decrease in catalase, but only a change in catalytic activity due to an increase or decrease in the acidity of the hydrogen peroxide. The following experiments were carried out in an attempt to clear up this phase of the problem. It should be said in this connection that most of these experiments had already been performed time and again in connection with our previous work on catalase, but they were not thought of sufficient importance to publish.

Twenty-five cubic centimeters of blood were taken from the external jugular of a dog and defibrinated. It was found that defibrination had

little or no effect on the catalase content of the blood. The abdominal wall of the dog was opened while the dog was under ether and 10 grams per kilo of sodium carbonate dissolved in 75 cc. of water per kilo were introduced into the upper part of the small intestine. The wound was then sewed up and the ether discontinued. Ninety minutes later 25 cc. more of blood were drawn from the jugular and defibrinated. The blood drawn before the introduction of the carbonate will be referred to hereafter as normal blood, while that drawn 90 minutes after the introduction of the carbonate will be referred to as carbonate blood. The catalase content of 1 cc. of the normal blood and of the carbonate blood was determined using slightly acid hydrogen peroxide, and it was found that the carbonate blood liberated about 30 per cent more oxygen than the normal blood. If this increase in the amount of oxygen liberated by the carbonate blood be due to the neutralization of the acid of the peroxide, thus removing the inhibiting effect of the acid, then the difference in the amount of oxygen liberated should be greatly decreased or disappear when neutral or slightly alkaline hydrogen peroxide is used. Catalase determinations of this same sample of defibrinated dog's blood were accordingly made, using neutral and slightly alkaline hydrogen peroxide. It was found that the carbonate blood liberated about 24 per cent more oxygen than the normal blood. This experiment would seem to show that only a very small per cent (6 per cent) of the increase in oxygen liberated by the carbonate blood was due to the neutralizing effect of the carbonate blood, while the remaining 24 per cent was due to an actual increase in catalase.

Further experiments bearing on this point of the following kind were carried out. Two cubic centimeters of blood were taken from the jugular of a dog. The catalase content of 1 cc. was determined immediately, while the other cubic centimeter was shaken with 10 cc. of 0.9 per cent of sodium chloride and placed in a water bath at 80°C. until the enzyme was destroyed. This tube was shaken at intervals to prevent the formation of a firm coagulum. Ten grams per kilo of sodium carbonate were introduced into the intestine of this animal and 90 minutes later another 2 cc. of blood were drawn from the jugular. The catalase of 1 cc. of this was determined immediately, while the other cubic centimeter was added to salt solution and heated at 80°C. for the same time that the control had been. The amount of oxygen liberated from hydrogen peroxide by the cubic centimeter of normal blood was 120 cc., while the carbonate blood liberated 168 cc. The heated cubic centimeter of normal blood as well as the heated cubic

centimeter of carbonate blood was added to slightly acid hydrogen peroxide. One cubic centimeter of blood from the same sample was added to each of these bottles and the amount of oxygen liberated was determined. Slightly more oxygen was liberated from the peroxide containing the heated carbonate blood than from that containing the heated normal blood. This result was the same as was obtained in the preceding experiment in that it shows that the neutralizing effect of the carbonate blood is responsible for a very small part of the increase in oxygen liberated by the blood after the introduction of sodium carbonate into the alimentary tract when slightly acid hydrogen peroxide is used. It should be mentioned in this connection that slightly acid hydrogen peroxide was not used in the experiments on the effect of acids, alkalies and salts reported in this paper, but neutral or slightly alkaline hydrogen peroxide.

Ten cubic centimeters of dog's blood were drawn from the jugular before as well as 90 minutes after the introduction of sodium carbonate into the alimentary tract and defibrinated. The carbonate blood liberated about 18 per cent more oxygen than the normal blood. Both of these samples were introduced into dialyzing tubes made of collodion and dialyzed against 2 liters of 0.9 per cent sodium chloride for 18 hours. At the end of this time these two samples were removed from the dialyzing tubes and made equal in amount by the addition of 0.9 per cent sodium chloride. Catalase determinations were made using 1 cc. from each of these samples, and it was found that dialyzing decreased very slightly the difference in the catalytic activity of the two samples. It had been previously found that catalase did not dialyze through these membranes to any appreciable extent. If the increase in the catalytic activity of the carbonate blood were due to the presence of the alkali in the blood, it would seem that dialyzing should have done away with the difference in activity between the normal and the carbonate blood or greatly decreased it. It might be objected, however, that the carbonate in the blood was in some way fixed and rendered non-dialyzable. Hence, the following experiments were carried out to determine if this objection were valid.

Ten cubic centimeters of blood were drawn from the jugular before as well as after the introduction of 10 grams per kilo of sodium carbonate into the intestine of a dog. It was found that the carbonate blood liberated 16 per cent more oxygen than the normal blood from neutral hydrogen peroxide. These two samples of blood were ashed at a temperature too low to decompose sodium carbonate, and the ash of

each was introduced into bottles containing equal quantities of neutral hydrogen peroxide. One cubic centimeter of blood taken from the same sample was added to each of these bottles and the amount of oxygen liberated from each was determined. It was found that the bottle containing the ash of the carbonate blood liberated practically the same amount of oxygen as that containing the ash of the normal blood.

If the increase in catalase after the introduction of alkalies into the intestine were due to the neutralizing effect on the acid of the hydrogen peroxide, then the introduction of acids into the alimentary tract should decrease the catalytic activity of the blood. In figure 2 it may be seen that the introduction of acids as well as acid salts increased the catalase of the blood instead of decreasing it. Furthermore, if alkalies when introduced into the alimentary tract of an animal increase catalytic activity by neutralizing the acid of the hydrogen peroxide, then the catalytic activity of the blood of the portal should be increased parallel with that of the liver. In figure 2 it may be seen under *ammonium carbonate*, *sodium carbonate* and *sodium phosphate* that such is not the case, but that the catalase of the liver blood is increased much more rapidly than that of the portal. This was interpreted to mean that these substances produced an increase in catalase by stimulating the liver to an increased output of this enzyme.

Determinations similar to the preceding were carried out using sodium phosphate instead of sodium carbonate with practically the same results. From these experiments the conclusion may be drawn that while a very small percentage of the increase in the power of the blood to decompose hydrogen peroxide after the introduction of alkalies and alkaline salts into the alimentary tract of animals is due to an increase in the catalytic activity when acid hydrogen peroxide is used, by far the larger part of the increase is due to an actual increase in catalase brought about by the stimulation of the alimentary glands to an increased output of this enzyme. It might be well to mention again in this connection that neutral or slightly alkaline hydrogen peroxide was used in the experiments reported in this paper on the effect of acids, alkalies and salts on the blood catalase, hence none of the oxygen liberated in these experiments can be attributed to the neutralizing effect of the alkalies.

Chvostek (9) showed that when hydrochloric acid was administered to a rabbit it decreased oxidation, while Lehmann (10) showed that an alkali, such as sodium carbonate, increased oxidation. In figure 1 it may be seen that the administration of hydrochloric acid to rabbits

produced a decrease in catalase, which is offered in explanation for the decreased oxidation as observed by Chvostek. The following experiment will show that the decrease in the blood catalase after the administration of hydrochloric acid to the rabbit is due in part to the inhibiting effect of the acid on the catalase, and in part to the direct destruction of the enzyme.

Ten cubic centimeters of blood were drawn from the jugular of a large rabbit and defibrinated. Two grams per kilo of hydrochloric acid dissolved in 75 cc. of water per kilo were introduced into the stomach of the animal. Ninety minutes later another 10 cc. of blood were drawn and defibrinated. The blood taken before the introduction of the acid will be referred to hereafter as normal blood and that after the introduction of the acid as acid blood. Catalase determinations were made of these two samples of blood and it was found that the acid blood liberated about 15 per cent less oxygen than the normal blood. One cubic centimeter of each of the samples was added to tubes containing 10 cc. of 0.9 per cent sodium chloride and heated at 80°C. until the catalase was destroyed. The heated normal blood as well as the heated acid blood was added to neutral or slightly acid hydrogen peroxide and catalase determinations were made using the normal blood. It was found that the bottle containing the heated acid blood liberated 10 per cent less oxygen than that liberated by the bottle containing the heated normal blood. This is interpreted to mean that of the 15 per cent decrease produced by the introduction of hydrochloric acid into the stomach of the rabbit, 10 per cent was due to the inhibiting action of the acid while the remaining 5 per cent was due to the direct destruction of the enzyme.

In figure 2 it may be seen that the introduction of hydrochloric acid into the intestine of a dog increased the blood catalase instead of decreasing it, as was found to be the case with the rabbit in figure 1. It may be seen further in figure 2 that ammonium chloride is a very effective stimulant to catalase production, 0.9 per cent per kilo being sufficient to bring about a great increase in this enzyme. Since the dog is a carnivorous animal, a relatively large amount of ammonium chloride is formed, when hydrochloric acid is administered, in the neutralization of this acid by ammonia, whereas the rabbit being a herbivorous animal, a relatively small amount is formed. Hence the increase in catalase after the introduction of hydrochloric acid into the intestine of a dog is attributed to the stimulating effect of the ammonium chloride on the liver giving an increased output of this enzyme.



Grafe (11) found that ammonium chloride increased oxidation in the rabbit. Lusk (12) showed that the ingestion of an amino acid, such as glycocoll, increased oxidation in the dog. The increase in catalase with resulting increase in oxidation after the ingestion of glycocoll is attributed to the stimulation of the liver by the glycocoll as well as by the ammonium carbonate and acetic acid resulting from the deamination of the glycocoll.

Further evidence that catalase is probably involved in the oxidative processes is found in the following observations. Müller-Thurgau (13) found that the respiratory metabolism is greatly decreased in potatoes kept for several days at 0°C., that it is greater at the beginning than at the end of the rest period, and also that it is greater in large mature potatoes than in small immature potatoes. Appleman (14) found that there was an increase in catalase in potatoes under the same conditions that Müller-Thurgau had found an increase in the respiratory metabolism and a decrease in catalase where he had found a decrease in metabolism. Appleman also found that there occurred an increase in catalase in the greening as well as the sprouting of potatoes parallel with the increase in the respiratory metabolism, while the oxidases were not increased and, in fact, were slightly decreased.

Hasselbalch (15) found that oxidation or metabolism is very low during the first month of life, and Magnus-Levy and Falk (16) that it is high during childhood. As a result of the work of these three observers and of Bailey and Murlin (17), Murlin and Hoobler (18), Howland (19), Benedict and Talbot (20), Benedict, Emmes, Roth and Smith (21), Palmer, Means and Gamble (22) and others, it is now considered that oxidation or metabolism is low during the first month of life, high during childhood and low after the onset of old age. Battelli and Stearn (23) found that the catalase content of most of the tissues and particularly of the liver of newly-born pigs is lower than the corresponding tissues of the mother, but that the catalase activity rapidly increased until at the end of the seventh or eighth day it was as high as that of the adult. We repeated and confirmed these observations, using the dog and newly-born puppies. We also determined the catalase content of the tissues of puppies that were about ten weeks old and found that the tissues generally were richer in catalase than those of the mother. The catalytic activity of the liver, for example, of the ten-week-old puppies was about 30 per cent greater than that of the liver of the mother. The catalase was determined by adding 1 gram of the hashed tissue, that had been washed free of blood to hydrogen peroxide and

the amount of oxygen liberated in 10 minutes was taken as a measure of the amount of catalase.

Warburg (24) found that during the process of fertilization, oxidation was greatly increased in the sea-urchin egg. Winternitz and Rogers (25) showed that the unfertilized hen's egg showed no catalytic activity even after prolonged incubation, whereas the incubated fertilized egg rapidly acquired the power of decomposing hydrogen peroxide. Doubtless if Winternitz and Rogers had determined the intensity of oxidation in the fertilized hen's egg, they would have found that it increased parallel with the increase they observed in catalase, and if Warburg had determined the catalase content of the fertilized sea-urchin egg, he would have found that this enzyme increased parallel with the increase he observed in oxidation.

J. Loeb (26) attributes the development of the fertilized egg to the increase in oxidation, and the increase in oxidation to a change in the cortex of the egg which makes the entrance of oxygen, and hence oxidation, possible, while R. Lillie (27) holds that the cortical layer of the unfertilized egg prevents the diffusion of carbon dioxide from the egg and that this carbon dioxide prevents oxidation and hence development. A more plausible explanation for the increased oxidation or metabolism in the fertilized egg, and hence for the development of the egg, would seem to be that the spermatozoön furnishes a substance which stimulates the egg to an increased formation of catalase. Further evidence in support of this view is afforded by the fact that the very same chemicals (amines, alkalies, acetates, butyric acid, etc.) which Loeb found would bring about increased oxidation and artificial parthenogenetic development of the egg, we found, when introduced into the alimentary tract of animals, stimulated the alimentary glands, particularly the liver, to an increased output of catalase with resulting increase in oxidation.

#### SUMMARY

1. The increase in oxidation produced by the introduction of an alkali such as sodium carbonate or phosphate into the alimentary tract of an animal is attributed to the increase in catalase brought about by the stimulation of the alimentary glands, particularly the liver, to an increased output of this enzyme.

2. The decrease in oxidation after the administration of an inorganic acid, such as hydrochloric, to rabbits is attributed to a decrease in catalase brought about by the inhibiting effect of the acid and by the

direct destruction of the enzyme. The increase in oxidation following the ingestion of organic acids, such as acetic, propionic and butyric, is attributed to an increase in catalase.

3. The increase in oxidation after the ingestion of the amino acids is attributed to the stimulation of the liver to an increased output of catalase by the ammonium carbonate and the organic acids resulting from the deamination of the amino acids, as well as by the amino acids themselves. The increase in catalase after the introduction of hydrochloric acid into the intestine of the dog is attributed to the stimulation of the liver to an increased output of this enzyme by the ammonium chloride formed in the neutralization of this acid by ammonia.

4. The increased oxidation in the sprouting of potatoes and the germination of grain with resulting increase in metabolism and development is attributed to an increase in catalase.

5. It is suggested that the low respiratory metabolism of the ovum before fertilization may be attributed to the low catalase content of the egg, while the increase in the respiratory metabolism after fertilization with resulting development may be due to an increase in catalase brought about by the stimulation of the egg by the spermatozoön to an increased production of catalase. Similarly the relatively low metabolism of the newly-born may be attributed to the poorness of the tissues in catalase, due to the small output of this enzyme from the liver, while the high metabolism characteristic of childhood and youth is a result of the richness of the tissues in catalase brought about by a large output of this enzyme from the liver.

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# ON THE PERMEABILITY OF THE PLACENTA FOR ADRENALIN IN THE PREGNANT RABBIT AND ALBINO RAT<sup>1</sup>

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## INTRODUCTION

At the present time the hormones are of great interest to many investigators. Not only the specific character of each hormone, but the interrelations between them give rise to very interesting problems and are the subject of many discussions. Under normal conditions the interrelation of the several hormones is self-regulated so as to maintain the physiological balance and keep the animal normal. Whenever this equilibrium is disturbed, there occurs a compensatory formation of hormones in several endocrine glands which tends to bring about a readjustment. Sometimes in consequence of this forced activity histological changes in these glands are more or less demonstrable.

During pregnancy the corpus luteum elaborates its hormone actively and consequently calls into responsive action, it is said, some of the other endocrine glands, as the thyroid, hypophysis, suprarenals, etc. Meanwhile, the ductless glands of the fetus develop to a certain degree and begin their function as endocrine organs and their products, including the hormones of the sex glands, are present in the fetal circulation. In multiparous mammals there may be several fetuses of both sexes in one uterus. Thus one pregnant animal incloses several hormone systems in her body, but in deciduate mammals these hormone systems are separated in a measure from each other by the placenta.

From such considerations the important question inevitably arises whether the placenta is permeable for the hormones or not. If the placenta is permeable, there will be a constant exchange of hormones

<sup>1</sup> The observations on the rabbits were made at the Zoölogical Laboratory of the University of Chicago. I wish to acknowledge my obligation to Prof. F. R. Lillie who gave me the best of laboratory facilities and gave me also many helpful suggestions.

not only between mother and fetus, but also between the fetuses themselves, though indirectly, by the intermediation of the mother's circulation, so that the male hormone, for example, will be delivered to the mother and the female fetus, and the hormone of the mother and the female fetus to the male fetus. Thus the interrelation of these hormones or hormone systems becomes much more complicated in pregnancy than in the non-pregnant state. From this point of view, the problem of the permeability of the placenta is very important, not only for the development of the fetus but also for the differentiation of sex.

For the solution of this question it seems to be important as a first step to learn whether a hormone administered to a pregnant animal can be transferred to the fetus through the placenta. As is well known, most of the hormones have not yet been isolated in a pure state; consequently their chemical character and constitution are still obscure, except in the case of adrenalin. Of course adrenalin may not represent the entire suprarenal hormone, but there is no doubt that adrenalin is an essential component of the suprarenal hormone, and we are justified therefore in taking adrenalin as a representative of the suprarenal hormone. If, in the first instance, we could get an experimental result by using such a well-characterized substance, we might apply the result to the next experiment and so gradually approach the solution of the problem.

With this idea in mind I have examined the effect on the fetus of the injection of adrenalin into a pregnant animal, using for this purpose rabbits and albino rats.

#### GENERAL PLAN OF EXPERIMENTS

The aim of my present experiment is simply to learn whether adrenalin administered to a pregnant animal is able to increase the adrenalin in the contained fetus.

At the end of pregnancy the suprarenal glands of the fetus elaborate their internal secretion and the fetal blood contains a certain amount of adrenalin. Therefore the proof of the presence of adrenalin in the blood of the fetus does not give a conclusive answer to the question. In order to say that the adrenalin administered to the mother has passed to the fetus, there must be a significant increase of adrenalin in the fetal blood as compared with the amount normally there.

There are several chemical and biological methods used to test the presence of adrenalin. In testing for adrenalin in blood the biological

method is considered in general to be better than the chemical. Among the biological methods that which depends on changes in blood pressure seems to be the most sensitive. But, because of its small size, it is almost impossible to measure the blood pressure of a fetus or a newborn individual of the ordinary laboratory animals. A considerable amount of blood is also required in order to examine the effect of adrenalin on the response of smooth muscle. Trendelenburg's method (1) also needs too much blood for my case. The frog-iris reaction requires only a few drops of blood and the manipulation is very simple, but the result is not always reliable.

It is a well-known fact that the parenteral administration of adrenalin causes hyperglycemia, and the animal is generally pretty sensitive to this test. If any increase of adrenalin occurs, it will be followed by hyperglycemia, without fail.

Thus, we can diagnose readily any increase of adrenalin by a quantitative determination of the sugar in the blood, and if we employ a micro-method, the blood sugar can be determined with a little blood, not exceeding the quantity that can be obtained easily from a fetus or several fetuses. Relying on this reaction, I injected adrenalin into pregnant animals and determined the blood sugar in the contained fetuses. Of course, the increase in sugar is not exactly proportional to the increase in the quantity of adrenalin, but I do not require that in my case because in my experiment it is necessary only to determine whether or not the adrenalin in the fetal blood has increased after the injection of adrenalin into the mother. Furthermore, it should be mentioned here that glucose, as is well known, very easily passes through the placenta and thus sugar in the mother's blood, increased by adrenalin, may increase the sugar in the blood of the fetus and thus obscure the real adrenalin effect on the fetus. To avoid such a result special precautions were taken, and these will be discussed later on.

For experimental material, as already mentioned, I used rabbits and albino rats, and made parallel experiments on both these species.

In my experiments the adrenalin used was that prepared by Parke, Davis & Co. The adrenalin was diluted with physiological salt solution before injection and the hypodermic injection was always made between the scapulae.

### *I. Experiments on rabbits*

As the rabbit is the most convenient and the most thoroughly studied animal in respect of adrenalin hyperglycemia, I took this animal first for my tests.

For the estimation of blood sugar in the rabbit I employed the Lewis-Benedict method (2) following the modification of Pearce (3). This method originally requires 2 cc. of blood for one determination, but it is almost impossible to obtain such an amount of blood from one rabbit fetus. Therefore I cut down the quantity of blood for one determination to one-fourth this amount, keeping all the other conditions—picric acid concentration, sodium carbonate concentration, etc., exactly as in the original formula. The sugar determination was made as follows:

To 2 cc. of water 0.5 cc. of blood was added. After the laking of the blood had taken place, 3.75 cc. of a saturated picric acid solution were added, and the solution agitated. To 3 cc. of the filtrate 1 cc. of saturated picric acid solution and 0.5 cc. of 10 per cent sodium carbonate solution were added. The mixture was heated in the autoclave for thirty minutes under a pressure of 20 pounds per square centimeter. Then the solution was made up to 5 cc. with distilled water and put into the colorimeter tube.

The Duboscq colorimeter is too big for my purpose and the Duboscq micro-colorimeter was not at hand, so I constructed a simple micro-colorimeter, using the parts of a burette and some other ordinary laboratory apparatus, and thus imitated the Duboscq instrument. This homemade micro-colorimeter gave me satisfactory results. Before proceeding to the experiment proper, I made some necessary preliminary tests.

*Adrenalin hyperglycemia in normal rabbits.* Concerning the increase in the blood sugar in rabbits after the adrenalin injection, there are many reports in the literature. I wished to determine this relation using the adrenalin which had been selected and the modified procedure of the Lewis-Benedict-Pearce method arranged for my own experiments.

Three rabbits were used for this purpose. During the night previous to the experiment the animals were kept without food. The next morning each animal received one subcutaneous injection of 5 cc. of 1:10,000 adrenalin. Just before the injection and after it at intervals of one-half, one, two, three and four hours the blood was taken from the ear vein of each animal. The quantity of sugar found in each specimen of blood is given in table 1. On the whole this result agrees with those previously reported.

*Adrenalin hyperglycemia in newborn rabbits.* There is no doubt that adrenalin causes hyperglycemia in adult rabbits. Now what influence will the adrenalin injection have on the blood sugar in newborn rabbits?

Though the cause of the adrenalin hyperglycemia is not yet clearly explained, it is generally considered as due to the mobilization of gly-



cogen deposited in the body tissues. Therefore if the newborn rabbit has any glycogen deposit in its body, hyperglycemia should be caused by adrenalin. Chipman (4) examined the glycogen of the fetal liver of the rabbit embryo and found that the glycogen appeared at the twenty-second day of gestation and increased rapidly and steadily in amount till the end of pregnancy. Lochhead and Cramer (5) made a quantitative determination of the glycogen in the fetal liver and the remainder of the fetal body in an age series of pregnant rabbits from the fourteenth day to the end of pregnancy.

According to them the fetal liver contains a trace of glycogen at the eighteenth day, though none can be demonstrated histologically till the twenty-second day. At the twenty-fifth day the percentage of glycogen in the liver rises for the first time above the glycogen percentage in the rest of the fetal body, and then there is a rapid increase till on the

TABLE 1  
*Adrenalin hyperglycemia in rabbits (0.5 mgm. of adrenalin administered subcutaneously)*

NUMBER	BODY WEIGHT	BEFORE INJECTION	PERCENTAGE OF BLOOD SUGAR HOURS AFTER INJECTION				
			$\frac{1}{2}$	1	2	3	4
	<i>grams</i>						
1	2360	0.08	0.12	0.26	0.26	0.23	0.16
2	2082	0.07	0.18	0.28	0.26	0.23	0.20
3	2187	0.10	0.20	0.31	0.28	0.25	0.21
Average .....		0.08	0.17	0.28	0.27	0.24	0.19

twenty-ninth day half of the fetal glycogen is stored in the liver. From these facts we can expect the sugar increase in the blood of newborn rabbits after adrenalin injection just as it occurs in the case of adult rabbits. With this expectation I tried the following experiments:

*Rabbit 1.* At full term of pregnancy;<sup>2</sup> body weight 2925 grams. Four fetuses were excised by Caesarean section. The body weight of the young varied from 34 to 38 grams. Immediately after removal one fetus was killed by bleeding from

<sup>2</sup> In my experiment the copulation of the rabbits was not watched, because it was not so necessary in my case to know the exact age of the fetus. My experiment required only rabbits in advanced pregnancy whose fetuses would have plenty of glycogen in their bodies and, at the same time, blood enough for a sugar determination. In rabbits the full term of pregnancy can be diagnosed without any difficulty. Several hours before the parturition they always begin to make a nest. This manoeuver is very evident and no one can miss it.

the neck while each of the others received 0.1 cc. of 1:10,000 adrenalin. At intervals varying from thirty minutes to two hours after the injection these were killed one after the other for blood specimens. The results of the sugar determinations are given in table 2.

*Rabbit 2.* At full term of pregnancy; body weight 2737 grams. Five fetuses were obtained by Caesarean section. The body weight of the young varied from 35 to 40 grams. The young were treated in the same way as those of rabbit 1. The percentages of blood sugar at different times are entered parallel with those for no. 1 in table 2.

This result shows that after the adrenalin injection the blood sugar increases in newborn rabbits as in the adults.

TABLE 2

*Blood sugar of newborn rabbits before and after the adrenalin injection (0.01 mgm. adrenalin subcutaneously)*

NUMBER	PERCENTAGE OF BLOOD SUGAR				
	Before injection	Hours after injection			
		$\frac{1}{2}$	1	$1\frac{1}{2}$	2
1	0.06	0.14	0.22		0.16
2	0.08	0.12	0.20	0.21	0.18

*The influence of adrenalin injected into a pregnant rabbit on the blood sugar in the contained fetus.* From the results of the preliminary experiments described above, we can expect an increase of sugar in the fetal blood after the injection of adrenalin into the mother, if the injected adrenalin is transferred to the fetus. As already mentioned, however, the increased sugar in the mother's blood is easily diffusible into the fetus. The increase of sugar in the fetal blood produced in this way is not only meaningless from the standpoint of my experiment, but tends to confuse any possible effect due to the passing of adrenalin into the fetus. Fortunately there is a certain space of time between the adrenalin injection and the distinct appearance of hyperglycemia. Therefore, if the fetus is removed from the mother before it is influenced in a marked way by the sugar in the mother's blood, such confusion will be practically avoided.

With these relations in mind, I made the following experiments.

Nine pregnant rabbits at full term were used. Their body weights varied from 2532 to 3112 grams. After a short period of inanition, from four to six hours, the animals each received one hypodermic injection of 5 cc. of 1:10,000 adrenalin. Then at varying intervals of

ten, twenty and thirty minutes after the injection the fetuses were removed by Caesarean section under ether. Immediately after removal from the mother, one fetus from each litter was killed by bleeding from the neck, while the others were kept warm till they were examined one after another for blood sugar at periods varying from twenty minutes

TABLE 3

*Blood sugar in newborn rabbits whose mother received 0.5 mgm. adrenalin before operation for delivery*

NUMBER	PERCENTAGE OF BLOOD SUGAR					
	Mother		New born			
	Before injection	At operation	Just after delivery	20 minutes after delivery	40 minutes after delivery	1 hour after delivery
<b>I. Fetuses removed from mother ten minutes after the adrenalin injection</b>						
1	0.10	0.10	0.10	0.09		0.08
2	0.06	0.07	0.06	0.07	0.06	0.05
3	0.09	0.11	0.08	0.08	0.06	
Average.....	0.08	0.09	0.08	0.08	0.06	0.07
<b>II. Fetuses removed from mother 20 minutes after the adrenalin injection</b>						
1	0.07	0.10	0.08	0.08	0.07	0.07
2	0.09	0.13	0.10	0.09		0.08
3	0.06	0.15	0.12	0.10		0.06
Average.....	0.07	0.13	0.10	0.09	0.07	0.07
<b>III. Fetuses removed from mother 30 minutes after adrenalin injection</b>						
1	0.09	0.15	0.12			0.06*
2	0.09	0.14	0.12		0.10	0.09†
3	0.06	0.18	0.15	0.12	0.12	
Average.....	0.08	0.16	0.13	0.12	0.11	0.08
<b>IV. Control, without adrenalin injection</b>						
1		0.09	0.08	0.08	0.07	0.07
2		0.08	0.08	0.08	0.07	0.07
Average.....		0.09	0.08	0.08	0.07	0.07

\* Two hours after the delivery the blood sugar = 0.07 per cent

† Two hours after the delivery the blood sugar = 0.07 per cent; three hours after the delivery = 0.05 per cent

to one or more hours after their delivery. Every blood specimen was used for a sugar determination.

Besides these, two pregnant rabbits at full term were used as control animals. Their body weights were 2435 and 2618 grams respectively. They were treated just as the test animals, but without any injection.

In all cases, in the tests as well as in controls, every placenta was carefully sliced and thoroughly examined for hemorrhages or other abnormalities. Where any pathological change appeared, the animal was omitted from the record. The results of the experiments are given in table 3—I, II, III and IV.

The data in table 3 (I to III) show a difference in the quantity of sugar in the fetal blood immediately after delivery which increases as the interval between the injection and the operation for delivery increases. Nevertheless, the blood sugar of newborn rabbits shows a tendency to decrease in the course of the first hours of extra-uterine life. The interpretation of these facts will be made later.

## *II. Experiments on albino rats*

As the next step in my work I repeated this series of experiments on albino rats. I was fortunately allowed to use pregnant and non-pregnant albino rats from the colony of these animals maintained by the Wistar Institute at Philadelphia.

In consequence of the smallness of this animal I employed the Epstein (6) micro-method for the blood sugar determination. This method requires only 0.1 to 0.2 cc. of blood for one determination and is based on the same principle as the Lewis-Benedict method, which is recognized as an admirable method and was employed by me for rabbits in the present study. The Epstein method does not give very accurate results. However, if it is carefully carried out, it is still fairly available for the usual biological tests, such as this present one, in which accuracy above a certain limit is not necessarily required. The matters to be attended to in connection with this method are the accuracy of the standard color solution and the purity of reagents, especially of the picric acid. Keeping these matters in mind, I got satisfactory results with this method.

In my experiments the rats were brought from the colony house in the morning and kept in the laboratory till the evening, without food, to avoid if possible the alimentary hyperglycemia. Then they received the experimental treatment.

In adult rats the blood was taken from the tail. The fetal blood was obtained from the neck, using two or three fetuses at one time. For one sugar determination 0.1 cc. of blood was always used. The colorimetric work was done almost always under artificial daylight, recommended by Gage (7).<sup>3</sup>

*The influence of fear and of ether narcoses on the blood sugar.* The rat, even when tamed, is not so gentle as the rabbit and is easily excited by unskilful treatment. Even a hypodermic injection or a sharp cut in the tail sometimes excites the animal greatly. Such an emotional disturbance affects the equilibrium of the blood sugar. Also the ether narcosis, when severe, has the same influence on the blood sugar. In working with such an excitable animal it is absolutely necessary to know first, how much influence these factors have on the blood sugar, because either agitation or narcosis was unavoidable in making the injection and obtaining the blood. To solve this question, I made the following tests with two rats: rat 1, male, body weight 182.4 grams, and rat 2, female, body weight 165.8 grams.

Both of these were roughly treated with the forceps, making them very angry. Each was then wrapped up in a towel and the blood taken by cutting the tail. To compare with these results samples of blood were taken from rat 3, male, body weight 174.6 grams; rat 4, male, body weight 126.9 grams; and rat 5, female, body weight 132.4 grams.

These animals had been treated very gently and carefully; wrapped in a towel, placed on the table softly, without causing any marked excitement, and the blood obtained by cutting the tail.

Finally rat 6, female, body weight 135.3 grams; rat 7, female, body weight 143.6 grams; rat 8, male, body weight 164.7 grams; rat 9, male, body weight 147.8 grams, were lightly etherized and the blood was taken in the same manner from the tail.

The blood sugar determination for each animal gave the results entered in table 4.

<sup>3</sup> The growth of the fetus of the albino rat becomes rapid toward the end of pregnancy. Parturition occurs usually at night. I made my tests therefore almost always at night in order to get the fully-grown fetus, and to obtain as much blood as possible. Thus I was obliged to make the color tests under artificial daylight, because the glucose in the solution diminishes in its reducing power with time and the color of the produced picramate fades pretty rapidly. In my experience the artificial daylight is rather better than natural daylight, if the background is not homogeneous, as is usually the case in cities or towns, where buildings are crowded around the laboratory.

This result shows that the effect of agitation is distinct, while the ether narcosis has no marked influence on the blood sugar, when, as in my case, the narcosis is neither deep nor long.

TABLE 4  
*Blood sugar in excited, not excited, and lightly etherized rats*

RAT NUMBER	SEX	BODY WEIGHT	MENTAL CONDITION	ETHER NARCOSIS	BLOOD SUGAR PER CENT	AVERAGE PER CENT
1	♂	182.4	Agitated	—	0.22	0.21
2	♀	165.8	Agitated	—	0.20	
3	♂	174.6	Quiet	—	0.16	0.16
4	♂	126.9	Quiet	—	0.18	
5	♀	132.4	Quiet	—	0.15	
6	♀	135.3	Quiet	+	0.15	0.16
7	♀	143.6	Quiet	+	0.15	
8	♂	164.7	Quiet	+	0.17	
9	♂	147.8	Quiet	+	0.16	

Rats 6 to 9 were narcotized once more at intervals varying from thirty minutes to one hour after the first narcosis. The blood of each animal at that period gave a percentage of sugar as shown in table 5.

TABLE 5  
*Blood sugar of twice narcotized rats*

RAT NUMBER	PERCENTAGE OF BLOOD SUGAR		INTERVAL BETWEEN TWO NARCOSIS
	First blood	Second blood	
			minutes
6	0.15	0.15	30
7	0.15	0.14	60
8	0.17	0.16	30
9	0.16	0.16	40

As this table shows, the repeated ether narcosis has no apparent effect on the blood sugar of albino rats, provided the narcosis is neither deep nor long. So we can use ether narcosis without fear of complicating our results.

*Adrenalin hyperglycemia in normal albino rats.* In the following tests each animal received 0.5 cc. of 1:10,000 adrenalin subcutaneously. Just before the injection and at intervals varying from thirty minutes to two hours after the injection, the blood was taken from the tail

under light ether narcosis. In the control animals 0.5 cc. of physiological salt solution was injected in the place of the adrenalin. The result of the sugar determinations is given in table 6.

TABLE 6

*Adrenalin hyperglycemia in albino rats (0.05 mgm. adrenalin injected subcutaneously)*

RAT NUMBER	SEX	BODY WEIGHT	PERCENTAGE OF BLOOD SUGAR				
			Before injection	Hours after injection			
				$\frac{1}{2}$	1	1 $\frac{1}{2}$	2
Controls							
1	♂	grams 148.7	0.15	0.16			
2	♂	151.6	0.16	0.16			
3	♀	159.1	0.17		0.17		
4	♀	141.8	0.16		0.16		
5	♂	123.6	0.16			0.18	
6	♂	170.1	0.17			0.15	
7	♂	177.3	0.16				0.16
8	♀	132.2	0.18				0.16
Average.....			0.17	0.16	0.17	0.17	0.16
Tests							
1	♂	130.4	0.13	0.18			
2	♂	165.5	0.16	0.19			
3	♂	136.8	0.14	0.18			
4	♀	143.2	0.16	0.19			
5	♂	155.6	0.15	0.18			
6	♀	120.8	0.15		0.20		
7	♀	139.3	0.15		0.22		
8	♂	148.7	0.19		0.21		
9	♂	177.1	0.19		0.31		
10	♂	158.1	0.19			0.31	
11	♀	114.3	0.15			0.23	
12	♂	139.2	0.14			0.20	
13	♂	121.8	0.15			0.24	
14	♀	146.3	0.15				0.21
15	♂	167.2	0.17				0.21
16	♂	186.1	0.15				0.19
17	♀	131.5	0.16				0.20
Average.....			0.16	0.18	0.24	0.25	0.20

Table 6 shows that in albino rats the blood sugar rises slowly after the adrenalin injection, reaching its maximum after one or one and a half hours. Though the percentage of the blood sugar at the highest point of the hyperglycemia is not much less in rats than in rabbits, the increase of the blood sugar is not so apparent as in rabbits, owing to the high percentage of normal blood sugar in the rats. In spite of that difference the albino rat is still a fairly available animal for my purpose.

*Adrenalin hyperglycemia in newborn albino rats.* The fetuses of three pregnant rats were removed by Caesarean section at full term.<sup>4</sup>

Immediately after delivery by operation two or three fetuses from each litter were used for the first blood specimen, while each of the others received a hypodermic injection of 0.05 cc. of 1:50,000 adrenalin, and then were kept in an incubator till they were killed at varying intervals of from thirty minutes to one and a half hours after the injection.

The blood sugar determination gave the results shown in table 7.

TABLE 7

*Blood sugar of newborn albino rats before and after the adrenalin injection (0.001 mgm. adrenalin administered subcutaneously)*

RAT NUMBER	PERCENTAGE OF BLOOD SUGAR			
	Before injection	Hours after injection		
		$\frac{1}{2}$	1	$1\frac{1}{2}$
1	0.14	0.18	0.20	
2	0.14	0.17		0.21
3	0.15		0.20	0.20
Average.....	0.14	0.18	0.20	0.21

These results indicate that the newborn albino rats also show a distinct reaction to adrenalin by an increase of the blood sugar. Therefore we can reasonably expect an increase of blood sugar in a fetus after the injection of adrenalin into the mother, if the injected adrenalin is transferred to the fetus, and this sugar increase should continue for one or more hours after the injection, regardless of the removal of the fetus from the mother.

<sup>4</sup> The full term of pregnancy of the rat can be diagnosed by palpation. The growth of the rat fetus in the last days of intra-uterine life is very rapid. The difference between the size of the fetus on the last day and on the day before is quite distinct. Therefore diagnosis of the last day of pregnancy is not very difficult after a little practice.



*The influence of the adrenalin injected into pregnant albino rats on the blood sugar of the fetuses.* For this series of experiments twelve pregnant rats at full term were used, nine as tests and three controls. Their

TABLE 8

*Blood sugar of newborn rats whose mother received 0.05 mgm. adrenalin before operative delivery*

NUMBER	PERCENTAGE OF BLOOD SUGAR					
	Mother		New born			
	Before injection	At operation	Just after delivery	20 minutes after delivery	40 minutes after delivery	1 hour after delivery
I. Fetuses removed from mother 10 minutes after injection						
1	0.13	0.13	0.13	0.12	0.12	
2	0.14	0.14	0.15	0.13		0.11
3	0.14	0.14	0.14		0.11	0.12
Average.....	0.14	0.14	0.14	0.13	0.12	0.12
II. Fetuses removed from mother 20 minutes after injection						
1	0.16	0.20	0.16	0.15		0.13
2	0.15	0.18	0.16	0.15	0.13	
3	0.15	0.17	0.16		0.12	0.13
Average.....	0.15	0.18	0.16	0.15	0.13	0.13
III. Fetuses removed from mother 30 minutes after injection						
1	0.17	0.20	0.18		0.14	0.15
2	0.14	0.19	0.17	0.16	0.13	
3	0.15	0.21	0.17	0.15		0.12
Average.....	0.15	0.20	0.17	0.16	0.14	0.14
IV. Controls: without adrenalin injection						
1		0.12	0.12	0.11		0.09
2		0.14	0.14		0.13	0.13
3		0.15	0.14	0.14	0.13	
Average.....		0.14	0.13	0.13	0.13	0.11

body weights varied from 153.6 to 215 grams. Each test animal received one hypodermic injection of 0.5 cc. of 1:10,000 adrenalin. At an interval of ten, twenty or thirty minutes after the injection, all the

fetuses were removed from each of the group of three animals under light-ether narcosis. Just before the injection and the operation respectively, the maternal blood was taken from the tail for the determination of sugar.

Concerning the fetuses: two or three fetuses from each litter were killed for the first fetal blood, immediately after delivery, while the others were kept in an incubator till killed for further blood specimens, at intervals of twenty, forty and sixty minutes after delivery.

The control animals received no injection. Otherwise they were treated in the same way as the test animals. The results of sugar determination for each blood specimen are given in table 8—I, II, III and IV.

If we compare these results on albino rats with those on rabbits, we find a striking similarity between them. Just after delivery the blood sugar of the fetuses is highest in those which remained longest in the mother's body after the adrenalin injection, and vice versa. In all cases, however, the fetal blood sugar decreases to normal or even to a subnormal value in the course of the first hour of extra-uterine life.

#### GENERAL DISCUSSION

My preliminary experiments show that in both the rabbit and the albino rat the fetuses after removal from the mother distinctly respond to adrenalin as do the adults by an increase in their blood sugar. Therefore, if the increased adrenalin in the maternal blood is able to raise the adrenalin concentration in the fetal blood, the adrenalin injection into a pregnant animal will increase the adrenalin in the fetal blood and consequently increase the fetal blood sugar.

In my experiments, however, there was no indication of an increase of adrenalin in the fetal blood after the injection of adrenalin into the mother. At the moment of delivery the blood of the fetus, which had been left in the mother's body for twenty minutes or more after the injection, showed a little increase of sugar. This slight hyperglycemia of the fetus might be taken erroneously as an effect of increased adrenalin in the fetal circulation. But such is not the case. If we once compare the percentage of sugar of this first fetal blood with that of the maternal blood at the moment of removal of the fetuses, we shall find a striking parallelism between them. Where the content is high in the mother it is also high in the fetus, though always a little lower absolutely than in the mother. Furthermore, we find that the blood

sugar of the fetus, in all cases, gradually decreases in the course of the first hours of its extra-uterine life, both in the tests and in the controls.

This is in contradiction to the course of the hyperglycemia of the fetus, which received an adrenalin injection immediately after delivery. The hyperglycemia caused by addition of adrenalin continued to increase for at least one hour after the animal received adrenalin. Therefore, if this hyperglycemia of the fetus were really caused by an increase of adrenalin in the fetal circulation, the blood sugar should continue its increase even after birth, because the fetus was removed from the mother at least within the first half of the supposed period of increment.

From such considerations we naturally come to the conclusion that adrenalin cannot be increased in the fetal circulation by injection of it into the mother; in other words, adrenalin injected into the pregnant animal does not pass through the placenta to the fetus. The high content of sugar in the fetal blood at the moment of birth is to be explained as a result of the diffusion of the increased sugar in the maternal blood toward the fetus.

To explain this negative result we must consider first the character of adrenalin, which is said to be easily destroyed in the living tissue under certain conditions. Numerous investigations regarding the fate of injected adrenalin in the animal body have been reported. In most of these investigations adrenalin was injected directly into the circulation and the effect of the drug on the blood pressure or on the tonus of the blood vessel was used as an indicator of its presence. The rise of the blood pressure after the intravenous injection of the drug lasts only a short time—thirty seconds to two to three minutes, according to the dose applied. Though this rapid cessation of the action of adrenalin on the blood pressure seems to be in favor of rapid destruction of the drug, we are far from having data that would justify us in supposing that the fall of blood pressure indicates the disappearance of adrenalin from the circulation.

Weiss and Harris (8) injected adrenalin and observed the circulation in the swimming membranes of the frog on both sides, while the arteria iliaca on one side was compressed. After the contraction of vessels on one side had completely relaxed, the vessels on the other side began to contract, when the ligature was released. In cats the blood, which no longer gave the adrenalin effect on the vessels, was however able to raise the blood pressure in the other animal, when it was injected. Again Miller (9) affirmed that the active substance does not disappear from the blood, and the blood of a rabbit, which has received a dose

of adrenalin, will cause a typical rise of blood pressure if injected into another rabbit, although the effect upon the first animal may have disappeared. Kahn (10) detected adrenalin in the blood by means of Trendelenburg's frog vessel preparation after the complete disappearance of adrenalin effect on the blood pressure of the injected animal, when the dose used was not too small.

Though there are many contradictory reports, these investigations just cited justify us in assuming that the intravenously injected adrenalin remains in the circulation even after the cessation of its effect on the blood pressure.

Moreover, many drugs, when subcutaneously injected, behave quite differently from those intravenously injected. The absorption is generally slower by subcutaneous injection than by intravenous injection. This is especially the case for adrenalin. The absorption of subcutaneously injected adrenalin is distinctly slowed by the contraction of the vessels around the point of injection. While the intravenous injection of adrenalin distinctly raises the blood pressure, the subcutaneous injection, at least in animals, does not raise the blood pressure, even in a large dose. After subcutaneous injection, therefore, we cannot use the effect of adrenalin on the blood pressure as an indicator of the drug. We have used here the effect of adrenalin on the sugar metabolism as an indicator, in the place of blood pressure.

Regarding the proportion of the quantity preserved to that destroyed when injection is made under the skin, we are unable to make a definite statement. Ritzmann (11) reports that 2 mgm. of adrenalin subcutaneously injected are equal to 0.4 mgm. intravenously injected, when measured by the amount of sugar appearing in the urine. From this fact we may suppose that about four-fifths of the subcutaneously injected adrenalin may be destroyed under the skin, before it enters the circulation. Even though the greater part of the injected adrenalin may undergo a destruction under the skin, it is improbable that this destruction occurs at the moment of injection, otherwise the absorption of one-fifth of the dose can be hardly explained. In reality this destruction does not seem to be so rapid. Embden and Fürth (12) report that 0.1 gram of suprarenin chloride disappears almost completely in two hours if added to 200 cc. of defibrinated beef blood, and if the mixture is constantly aerated at body temperature.

Furthermore, it is a wellknown fact that in man a subcutaneous injection of 0.5 mgm. adrenalin raises the blood pressure after 5 to 12 minutes. The maximal height continues for 2 or 3 hours and is then followed by a gradual fall.

Therefore it is reasonable to assume that by subcutaneous injection the drug is absorbed gradually and slowly by the circulation, and consequently the adrenalinemia will last a considerably long time, at least longer than that caused by an intravenous injection.

As stated already, I injected adrenalin hypodermically (0.5 mgm. for rabbit, 0.05 mgm. for albino rat) and examined the blood sugar of the fetus at intervals varying from ten to thirty minutes after the injection, but I found no indication of an increase of adrenalin in the fetal blood. In giving an explanation of this negative result, three factors are to be considered: first, the quantity of adrenalin administered; second, the duration of adrenalinemia of the mother animal; and third, the impermeability of the placenta for adrenalin.

Concerning the first factor there can be no doubt that the quantity administered was sufficient for my purpose, even on the assumption that nine-tenths or more of the injected drug is destroyed under the skin. Moreover, my experiment itself clearly proves that the quantity was sufficient, because every injection caused a strong hyperglycemia in the mother animal.

To answer the question as to how long the adrenalinemia of the mother animal lasted, is not easy. However, as mentioned above, we have reason to believe that the adrenalinemia caused by a subcutaneous injection of adrenalin lasts for a long time. In my experiments thirty minutes was the longest interval from the injection to the removal of the fetus. During this interval, the increased blood sugar of the mother had already passed to the fetus in a considerable amount, but no trace of adrenalinemia was to be seen in the fetus, despite the length of this interval, which ought to have been long enough for the passage of such a crystalline substance through the placenta.

It appears therefore that the quantity of adrenalin was enough and the interval for the transfer of adrenalin from mother to fetus was also sufficient. Consequently if adrenalin were able to pass the placenta, there must have been an adrenalinemia also in the fetus, but the results are absolutely negative. We find the explanation of this negative result in the third factor, the impermeability of the placenta for adrenalin. On the basis of these few experiments, of course, I do not dare to say that the placenta is absolutely impermeable for adrenalin, but it seems certain, at least, that we cannot increase adrenalin in the fetal blood by a subcutaneous injection of the drug into the mother animal. Whether or not a more severe and longer continued adrenalinemia of the pregnant animal can increase the adrenalin in the fetal blood, is still an open question.

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# THE CHARACTER OF THE SYMPATHETIC INNERVATION TO THE RETRACTOR PENIS MUSCLE OF THE DOG

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In recent years the retractor penis muscle has attracted considerable attention from anatomists and physiologists as it has served as an object for the study of the physiology of smooth muscle for which work it is especially well suited, on account of the simple arrangement of its fibers. A second reason which has aided in making it useful in physiological and pharmacological experimental work is its innervation which, as described, is simpler than that of most of the smooth muscle structures. As Fröhlich and Loewi (1) point out, some of the more complicated structures as, for example, the vessels and hollow organs possess a fourfold innervation while the simply constructed retractor muscle has only a double innervation.

The muscle was first described by Eckhard, but for our knowledge of its innervation we are indebted to Langley and Anderson (2), who found that the motor fibers for the muscle were contained in the lumbar sympathetics, the fibers running to the muscle chiefly in the nervus dorsalis penis. They state further (*loc. cit.*, p. 93) that they could find no satisfactory evidence of the presence in the lumbar nerves of inhibitory fibers for the external generative organs either in the male or in the female.

The inhibitory fibers they found came from the pelvic nerves being contained in the nervi erigentes. The evidence in favor of the correctness of this statement I have summarized elsewhere (3).

These views of the nerve supply of this muscle as described by Langley and Anderson have been accepted apparently by all subsequent workers upon this structure and the various text-books which refer to it quote the above mentioned work as their authority. Reference may be made to Fletcher (4) and to Schäfer (5); to De Zilwa (6), Fröhlich and Loewi (*loc. cit.*), and to Brücke (7), and finally to Bottazzi (8), who has carried out a very extensive research upon the physiology of smooth muscle using the retractor as the test object.

*Present research.* My own experiments with this muscle were carried out in connection with some investigations that were being made upon the pharmacology of the bladder and in an effort to explain the results which were obtained upon that organ. These results are described elsewhere (3).

Most of the work in that research was carried out upon the isolated muscle immersed in oxygenated Ringer's solution in the usual manner and maintained at a temperature of between 34.5°C. and 35°C., which temperature appeared to suit its activity best. Confirmatory experiments were carried out upon the intact dog. Pieces of the retractor muscle prepared in this way usually start up an automatic rhythm in from ten to thirty minutes and these movements will continue over long periods of time if the oxygen supply and the proper temperature are maintained.

If to the Ringer's solution in which the muscle is immersed, a drop or two of a solution of adrenalin chloride (1-1000) is added, the muscle promptly contracts in harmony with the well-known rule that adrenalin results are similar to those produced by sympathetic stimulation.

In connection with my experiments upon pilocarpine and other alkaloids upon the retractor, I found it necessary to paralyze the sympathetic endings in order to eliminate that structure as a possible point of attack for these alkaloids. For this purpose a small amount of ergotoxin was added to the Ringer's solution, and the muscle was allowed to remain exposed to it for a short time. To the muscle in fresh Ringer's solution adrenalin was added as before in order to test the completeness of the paralysis and now in place of the adrenalin producing a contraction or being inactive as was to be expected, the muscle responded by a marked relaxation. The most likely explanation of this phenomenon when taken into consideration with the action of adrenalin after ergotoxin elsewhere in the body was that inhibitory nerves were present in the sympathetic and that these are ordinarily masked by the more powerful motor fibers and are only able to demonstrate an action when the motor fibers are paralyzed. These results obtained with adrenalin after ergotoxin were confirmed upon other preparations of the isolated retractor with results entirely similar to those described above, but in order to prove the correctness of the explanation offered, it was necessary to repeat the experiments using the entire animal instead of the isolated muscle and testing at the same time the sympathetic nerve for a possible alteration in its reaction.



Accordingly a medium sized dog was anaesthetized with chloretone dissolved in oil and after anaesthesia was complete tracheal and venous cannulae were inserted. The abdomen was then opened and the hypogastric nerve was isolated and prepared for stimulation. The retractor muscle was next exposed and the peripheral end cut from its point of insertion and the muscle separated from its fascial covering. The peripheral end of the muscle was connected with a recording lever and the muscle itself protected from drying by a covering of absorbent cotton kept moist with warm salt solution.



FIG. 1

Fig. 1. Adrenalin upon the normal isolated retractor penis muscle. Lever moves down in contraction.

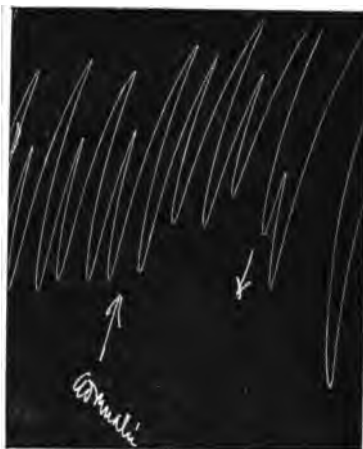


FIG. 2

Fig. 2. Adrenalin upon the isolated retractor muscle which has been subjected to action of ergotoxin. Lever moves down in contraction.

Injections of adrenalin and sympathetic stimulation, obtained as controls, were followed by the customary contractions. Ergotoxin was now injected into the jugular vein and the progress and extent of the paralysis was tested by repeated injections of adrenalin. It was found that the nerves were quite resistant to the action of the ergotoxin, three injections of ergotoxin being necessary before the motor effects of the adrenalin upon the blood pressure were obliterated. When this stage was reached not only did the retractor respond to the adrenalin by relaxation but electrical stimulation of the hypogastric was also followed by an elongation of the muscle thus demonstrating the presence of

inhibitory fibers in the sympathetic nerve to the retractor and bringing this structure more closely into harmony with those abdominal organs possessing a double innervation.



FIG. 3



FIG. 4

Fig. 3. Tracing of blood pressure in dog with effect of stimulation of sympathetic nerve upon the retractor muscle. Lever moves down in contraction.

Fig. 4. Blood pressure in same dog as shown in figure 3 with result of hypogastric stimulation on retractor muscle after ergotoxin has paralyzed the motor fibers. Lever moves up as muscle relaxes.

Figures 1 and 2 show the effect of adrenalin upon the isolated retractor muscle before and after ergotoxin, while figures 3 and 4 were obtained from the retractor muscle in an intact dog and show the effect of sympathetic stimulation before and after the use of ergotoxin.

SUMMARY

The sympathetic nerve to the retractor penis muscle of the dog contains inhibitory as well as motor fibers.

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## ENERGY EXPENDITURE IN HOUSEHOLD TASKS

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The expenditure of energy by an individual is influenced by a number of factors, among the more significant of which are age, size, sex and occupation. The effect of some of them is relatively constant, while that of others varies decidedly with circumstances. This is especially true with regard to occupation. The expenditure of energy in the performance of any task varies with the nature of the task and with the rate at which it is performed. If the effect which the several factors have had upon the transformations of energy in the body were known and the length of time each was effective, it would be possible to gauge the energy requirements of an individual in the various circumstances of his daily life. Furthermore, information of this sort is essential in estimating the amounts and kinds of food required by an individual to meet the needs of his body for energy.

For a number of years the work of the Office of Home Economics has included investigations of these various factors as affecting both men and women. The present article presents the results of some studies of the energy requirement for the performance of several household tasks. These experiments are part of an extended and comprehensive series which included not only measurements of the energy expenditure in the performance of the different kinds of tasks and of similar tasks under different conditions, but also the amount of time expended in such tasks under varying conditions of equipment, personnel and routine.

Variations in the transformation of energy in the body are accompanied by corresponding variations in the heat output, and the expenditure of energy by the body is estimated from measurements of the heat produced. Such measurements are made by means of a respiration calorimeter. A series of experiments, which has been referred to in reports of the Department of Agriculture (1), was made with one of

the calorimeters in the laboratory of the Office of Home Economics which was described in a publication of the Department (2). A report of the results of these experiments, which is now being prepared, was interrupted by the entrance of the investigator who conducted them into the military service. The experiments here reported are a continuation of the work, but with the respiration calorimeter somewhat modified to overcome difficulties that had been experienced in the course of the preceding series. The size of the chamber had been reduced to increase the reliability of the measurement of oxygen consumed by the subject, and the mass of metal in the structural framework of the apparatus had been removed because its presence introduced difficulties in securing accurate data regarding the heat conditions of the chamber whenever any change was made in the activities of the subject and consequently in the rate of production of heat within the chamber.

Briefly stated, the respiration calorimeter consists of a chamber (75 x 120 x 200 cm.) with air-tight walls, connected with devices for maintaining a current of air through it from which the water vapor and carbon dioxid eliminated by the subject within the chamber can be removed, and equipped with devices for carrying away and measuring the quantity of heat produced within the chamber. The subject of the experiments remains in this apparatus during the experimental period, resting or performing some specified task in accordance with the plan of the experiment. A comparison of the amount of energy transformed by the body during a given period of time while at complete rest and while doing the prescribed work is taken as an indication of the requirement of energy for the performance of the specified task.

With an apparatus of this sort energy expenditure may be determined in two ways, directly by calorimetric measurements of the heat produced by the body, and indirectly by computation from the amount of oxygen consumed and carbon dioxid produced by the subject. In the present experiments the two methods gave results that were practically identical. Only the figures obtained by the direct measurement of heat are here discussed as these are fundamentally the more accurate.

#### PLAN OF EXPERIMENTS

The subject for this series of experiments was a woman twenty-two years old, of rather thin and spare build, 5 feet 4 inches tall, and weighing 110 pounds (50 K) in uniform, her weight being taken at the end

of each experiment. The uniform, consisting of a "middy blouse" and skirt of cotton with very light underclothing, was the same in each experiment. The experimental period, which was always of two hours' duration, started at approximately the same time each day so as to obtain as far as possible the same physiological conditions. The subject ate no breakfast, but about 7:30 each morning she drank one cup of cocoa made uniformly of half a pint of cream, 2 teaspoonsful of cocoa and 1 teaspoonful of sugar. She arrived at the laboratory so as to enter the calorimeter about 10:30 a.m., and measurements of the experimental data started between 11:00 and 11:30. While in the calorimeter she followed a routine that was carefully planned previous to the experiment and performed all her work to the beat of a metronome, so that an exact count of the number of movements was possible. Therefore, a complete time-motion study is provided for each kind of work. The data thus obtained may be used for comparison with the energy required for any similar task actually performed in ordinary life where the rate of work may be either faster or slower. The series includes experiments with the subject at rest and performing various household tasks.

The tasks performed during these preliminary experiments were chosen partly because they represent some of the more common and typical household activities and partly because they seemed likely to indicate how experimental methods should be developed for this type of investigation, especially in the matter of reproducing actual working conditions within the calorimeter. In the experiments with washing no water was used because of the complicating effect it would have on the water vapor and heat measurements, but it is hoped to devise means of overcoming this difficulty in later experiments. The results of fifty-three tests (briefly noted in the report of the work of the Office of Home Economics, presented at the 11th annual meeting of the American Home Economics Association, Science Section, Chicago, June, 1918) (3), are summarized in table 1.

*Rest experiments.* The first measurements of energy expenditure by this subject were made in four rest experiments in which the external muscular activity was reduced to a minimum. For this purpose the subject rested easily in a comfortable position in a large swivel chair, and remained very quiet, hardly moving during the two hours comprising each rest experiment. The number of calories measured during each of the four experiments was very uniform, indicating that the expenditure of energy by this subject while sitting quietly was practically

constant. The average of the values for these four experiments is used as a basis of comparison with those for experiments in which work was performed, showing the increase in heat output resulting from the muscular activity involved in the performance of the work.

*Experiments with knitting, crocheting and hand sewing.* The measurements of the energy expended while knitting, crocheting and hand

TABLE 1

*Average energy (heat) output during experiments with a woman engaged in different household tasks*

CHARACTER OF EXPERIMENT	NUMBER OF EXPERIMENTS	HEAT ELIMINATION			
		Total per 2-hour period	Total per hour	Per hour per kilogram of body weight	Per hour for work alone
		calories	calories	calories	calories
Resting.....	4	121.4	60.7	1.22	
Knitting.....	4	141.2	70.6	1.42	10.1
Crocheting.....	5	138.0	69.0	1.39	8.3
Sewing					
Scalloping.....	2	140.8	70.4	1.43	10.2
Running.....	2	136.4	68.2	1.39	7.5
Hemming.....	2	139.6	69.8	1.45	10.3
Hemming and running.....	3	142.4	71.2	1.39	9.8
Darning.....	2	137.4	68.7	1.40	8.0
Average for sewing.....	11	139.6	69.8	1.40	9.4
Dressing infant (model).....	4	168.8	84.4	1.72	23.6
Sweeping floor.....	4	201.4	100.7	2.06	40.1
Washing floor.....	3	177.6	88.8	1.82	29.0
Washing towels.....	4	220.8	110.4	2.22	49.6
Ironing towels.....	6	171.6	85.8	1.75	24.3
Dishwashing					
Table 65 cm. high.....	2	181.6	90.8	1.85	30.0
Table 100 cm. high.....	4	190.2	85.1	1.74	24.4
Table 85 cm. high.....	2	162.2	81.1	1.65	20.3
Average for dishwashing.....	8	181.1	90.5	1.75	24.8

sewing, were all conducted with the subject comfortably seated in an ordinary bentwood chair, and all motions except those necessary for the work were reduced to a minimum.

The knitting was done on a sweater about half completed. The rate of work was 23 stitches per minute taken to the beat of a metronome.

In the crocheting experiments, fine cotton thread was used and a simple pattern chosen. The rate of the work was 32 stitches per minute.

The experiments with hand sewing included several types of work: *a*, making a plain unpadding scallop (blanket stitch) on the edge of a small piece of fine linen, at the rate of 18 stitches per minute; *b*, simple running on light cotton goods, 6 stitches being taken on the needle; one to one beat of the metronome, then the thread pulled through to four beats, with a total of 30 stitches per minute; *c*, hemming on light cotton goods, at a rate of 30 stitches to the minute; the thread being pulled all the way through after each stitch; *d*, darning light-weight cotton hose with a thread about 24 inches long.

As might be expected these tasks all entail a relatively small expenditure of energy, varying from 7 calories per hour for sewing with the running stitch to 10 calories per hour in hemming. The expenditure during knitting (10 to 11 calories per hour) is slightly higher than that during crocheting (8 to 9 calories), doubtless because the wool sweater which was handled in knitting weighed more than the cotton lace which was being crocheted, and because greater play of the hands and arms was required by knitting with fairly long needles than by the crocheting. Since in the sewing experiments the materials used were of approximately the same weight and ease of handling, the differences there are probably to be attributed to the extent of movement required; thus with the running stitch (7 calories per hour) the movement of drawing the thread to its full length was made only every 6 stitches or 5 times per minute, whereas with the hemming (10 to 11 calories per hour) it was made after each stitch or 30 times per minute. This is in line with the practical experience of needleworkers, who for most forms of plain sewing avoid an unnecessarily long thread as causing too much work of the arms. In later experiments it is planned to introduce sewing on articles of different types in order to learn how such factors as weight, shape and texture influence the energy expenditure.

*Experiments on dressing model of infant.* It was desirable that some observation be made on the energy output involved in caring for children and the task of dressing and undressing an infant was chosen for this preliminary test. Because of the difficulties in conducting the experiment with a young child, a full-sized model was substituted. The model was about the size of a year-old infant, but it weighed, dressed, only 2 kgm., whereas a child of this size would weigh about 8 to 10 kgm. The clothing of the model consisted of a band (no sleeves), diaper, shirt with sleeves, two underskirts (no sleeves), dress, knitted,



sack with sleeves, socks, booties and bonnet. The model was dressed and undressed seven times during each 2-hour experiment.

The energy output in this experiment, 23.6 calories per hour for work alone, is undoubtedly lower than would be required in the care of a living child, as a child of this size would be four or five times as heavy to lift and its movements would still further increase the energy output. These figures, therefore, represent only the labor involved in the act of removing and replacing the clothes plus the necessary lifting about and supporting of a 2-kilogram model.

Even the unduly low figure thus obtained (24 calories in round numbers per hour) was fully twice as great as that for such light tasks as sewing, similar to that required for dishwashing and ironing and about one-half that for washing clothes. This indicates that such work should be classed as a moderately heavy household task. A part of the program for experiments now being conducted is the measurement of energy expended in the care of living children and it is believed that the value of the results thus obtained will be sufficient to offset the difficulties involved.

*Experiments with sweeping and washing floors.* These tasks were included as representing what are usually considered rather hard forms of housework. The sweeping was done on the bare floor of the calorimeter. A long-handled broom was pushed forward on the floor, lifted and moved back. The rate was 38 complete strokes per minute.

In the floor-washing experiment, the motions of actual washing were simulated with a dry cloth and empty pail. The subject worked on her knees, giving 85 short rubs of the cloth on the floor in 50 seconds and wringing the cloth in the pail for 10 seconds.

When compared with such tasks as knitting and sewing, sweeping the floor would be classified as hard labor, the energy expenditure, 40.1 calories per hour for work alone, being four or five times as great. Plans have been made for experiments with various types of floor coverings. This work should give interesting results as to the relative resistance offered to the broom by other surfaces as compared with the smooth floor of the calorimeter, and the consequent variations in the energy required for sweeping.

The average of 29 calories per hour for energy expenditure during floor washing is believed to be considerably too low for usual work of this kind. Not only was the task slightly lightened by omitting water, which would add to the work of lifting and wringing the cloth, but the subject, who was unused to such labor, was not able to continue exerting

as much force as a professional charwoman would expend and her movements were those of light rubbing rather than hard scrubbing. It is planned to repeat the experiment with a stronger subject accustomed to such tasks.

*Experiments with laundry work.* In the experiments with laundry work, both washing and ironing were included. The work was done on towels 16 inches square.

In washing, the equipment consisted of a small galvanized iron tub and an ordinary scrubbing board. A towel was rubbed on the board 40 times in 30 seconds, wrung by hand for 15 seconds, then exchanged for another towel, 15 seconds being allowed for the exchange. This routine was repeated throughout the experiment.

The energy output in washing was 49.6 calories per hour for work alone; this is similar to that found for sweeping floors and like it must be classified as hard labor. It seems probable that the weight of the water in actual washing might increase the labor of moving the towel and also that wet articles might offer slightly more resistance on the scrubbing board and in wringing. Moreover in ordinary family washing the average weight and size of the articles handled are greater than those of the towels used in these tests, and would tend to increase rather than decrease the energy expenditure. The figures here given, therefore, represent less rather than more energy than would be expended for comparable work in actual laundering. It is hoped to devise some way to overcome the difficulties of using water in experiments with such types of work and to approximate more closely the conditions actually found in the household.

In the ironing experiment, the work was done with a 5-pound iron on a table of comfortable working height. The rate of work was 70 strokes of the iron in 50 seconds, and 10 seconds to change towels. A cold iron was used because of the complications which heat would cause in the measurements, but it is not probable that this omission would noticeably affect the amount of energy expended by the worker.

The energy expenditure in the ironing experiments, which in six experiments averaged 24 calories per hour, lies between that of the tasks classed as light work and those classed as heavy work and might be classed as moderate work.

*Experiments with dishwashing at tables of different heights.* In the dishwashing experiments a table of adjustable height was used so that studies might be made of the comparative amounts of energy expended when working surfaces of different heights were used. In one set of

observations the table was too low for comfort, in another, too high, while in a third it was set at what was regarded as the correct height for the worker.

The dishes, comprising 4 plates, 2 bowls, 2 teacups and 2 saucers, were placed in a pan, rubbed with a cloth, placed in a draining pan and then wiped. The motions were the same as in actual washing. Each dish was given 10 rubs, turned, and given 10 more, in time with a metronome, beating 130 times per minute, then 10 beats were allowed to change pieces. The work simulating drying was then performed at the same rate. The whole process was repeated twelve times per hour.

In the series of tests in which the table top was set too low for the comfort of this subject, the height from the floor to the top of the table was 65 cm. and to the top of the pan 78 cm. In the series in which it was too high, the top of the table was 100 cm. and the top of the dishpan 113 cm. from the floor. In the series in which the height was adjusted to the comfort of the subject the table top was 85 cm. and the top of the pan 98 cm. from the floor.

As regards the general question of energy expenditure the average figure of 24.8 calories per hour shows that washing table dishes is to be classed as moderate work, about midway between sewing and sweeping and washing. Somewhat more heat would no doubt be eliminated with heavier dishes (large platters, kettles, etc.) or ones which require harder rubbing than was given in these tests.

As regards the differences in the energy expended at tables of different heights, the figures show 21 calories per hour when the subject worked in a comfortable position, 25 calories when the pan was set so that her arms were raised during work, and 30 calories when she was obliged to bend over. It is hoped in the future to develop such experiments so that the oncome and effect of fatigue may be noted when similar tasks are performed under varying conditions.

#### SUMMARY

Fifty-three experiments on energy elimination during the performance of various household tasks were made, using a specially designed respiration calorimeter and a young woman subject. The results for such light tasks as sewing, crocheting, knitting, darning and embroidering, gave an average expenditure of 9 calories per hour more than that of the same subject sitting quietly in a chair; other tasks regarded as "harder work" than sewing, such as washing, sweeping and scrubbing

floors, caused an increased energy expenditure over the expenditure when at rest with the same subject, of about 50 calories per hour. Several other tasks studied gave results between these two figures: thus, ironing, dressing a child (life-size model) and dishwashing each requiring about 24 calories per hour.

During the experiment with dishwashing the height of the table used was varied, and a corresponding variation in energy expenditure was noted, a variation of 15 per cent in height of table causing an increase in energy expenditure of 20 per cent to 40 per cent. The observed increase of heat elimination well illustrates the importance of choosing equipment to "fit" the worker.

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# THE AMERICAN JOURNAL OF PHYSIOLOGY

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## AMPLIFICATION OF ACTION CURRENTS WITH THE ELECTRON TUBE IN RECORDING WITH THE STRING GALVANOMETER<sup>1</sup>

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Received for publication April 6, 1920

### INTRODUCTION

In the spring of 1916 Dr. H. B. Williams suggested the use of the electron tube to amplify those action currents in the nervous system which are too small to record with the string galvanometer alone. In the course of several researches in this laboratory we have encountered difficulties due to the extremely small excursions obtainable with the string galvanometer from the action currents in nervous tissue, notably in the activity of the spinal cord and in the motor nerve involved in the crossed extension reflex. The suggestion appeared therefore to offer possibilities of attacking physiological problems hitherto inaccessible.

Military duties prevented the development of this suggestion until the spring of 1919, when conditions in this laboratory became favorable for the work. Doctor Williams was still absent from his laboratory on duty; but the prospect of utility of the method made us feel justified in proceeding to develop it without waiting for his return and the collaboration we should have preferred. We wish to express our appreciation of his generosity in urging us to publish the work as our own, and to thank him for certain helpful suggestions in the later phases of the work since he returned to his laboratory.

There is nothing new in the idea of amplifying electric currents with the electron tube. Especially in the field of radio communica-

<sup>1</sup> A preliminary report has appeared; This Journal, 1920, li, 177 (proceedings).

tion, this has been standard practice for years. The usual method of observing the currents amplified by this device is with the telephone receiver. This method is not applicable to those problems in physiology which have led us to this work. In using the string galvanometer to record the amplified currents we are confronted with several new problems which do not enter into the telephone method. These problems are of practical importance to the physiologist who would use the method, and of some theoretical interest to the physicist. It has seemed worth while to carry out a good many experiments and to attempt to correlate them with theoretical expectation, in order to formulate, if possible, the correct method of obtaining the maximum amplification, or at least to outline the principles on which such a method must depend. We have therefore taken up some of the mathematical considerations involved although these may be of little interest to the physiologist who wishes merely to know how to use the method. The mathematical discussion may readily be skipped, and simple instructions still be found for obtaining very nearly the maximum possible amplification.

#### THE ELECTRON TUBE

The electron tube or thermionic amplifier (1), commonly known in this country as the audion, consists essentially of a vacuum tube containing a cathode in the form of a filament, heated to incandescence

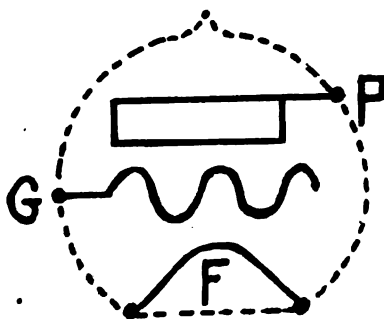


Fig. 1. Conventional diagram of electron tube elements. *F*, filament; *G*, grid; *P*, plate.

by the passage of a current, an anode in the form of a metal plate, and an auxiliary electrode in the form of a grid (2) placed between the anode and the cathode. A conventional and schematic representation of these elements is shown in figure 1; this is convenient for identification of the parts in circuit diagrams to follow.

When the filament (cathode) is incandescent it emits electrons into the space within the tube. If by means of a battery the plate (anode) is made positive with respect to the filament, these electrons under the influence of the electric field thus set up, travel to the plate and thus enable a current to flow from plate to filament within the



tube (according to the conventional designation of the direction of current flow). Under ordinary conditions of operation, more electrons are emitted by the filament than can be drawn to the plate by the voltage applied to it; a negative "space charge" results which repels electrons subsequently emitted and causes them to return to the filament. This space charge exerts a limiting effect on the current that passes from anode to cathode. If now a positive potential, with respect to filament, be applied to the grid, or auxiliary electrode, it will oppose the space charge and enable more current to flow in the tube; if a negative charge be applied to the grid it will still further limit the current. In this way the current in the tube may be regulated by variations in the potential of the grid. In a properly designed tube small variations in the potential of the grid may be made to produce large variations in the plate-to-filament current, and in this way the device acts as an amplifying relay, the energy of amplification being supplied by the battery which maintains the high potential of the plate.

#### METHODS IN GENERAL

The salient feature of our problem as compared with the ordinary uses of the electron tube was the necessity of protecting the delicate string of the galvanometer from the direct current used to maintain the high potential of the plate.

Telephone receivers are ordinarily placed directly in series with the plate and the high voltage battery, as is shown in figure 2. This would be impossible with a sensitive string galvanometer. With the Western Electric "D-tube," for instance, the optimum plate potential is 200 volts; under the most favorable working conditions this tube has a plate-to-filament resistance of about 100,000 ohms. Neither string nor telephones placed in series with this would add greatly to the total resistance of the circuit. Therefore, the current that would pass through the string would amount to about 2 milli-amperes. One hundredth of this current is as much as or more than it is safe to pass through a string of 2 or 3 micra diameter, the size best suited to nerve physiology. Even were it possible to pass the full plate current through the string without destroying it, to work under this condition would be impossible, for such a current would move the string well out of the field accessible to the beam of light required for projection.

Three methods have presented themselves for utilizing the amplifying energy of the plate battery without placing the string directly in the path of a damaging current. The first is to place the primary coil of a transformer in the plate circuit, and connect the galvanometer across the terminals of the secondary coil of the transformer (see

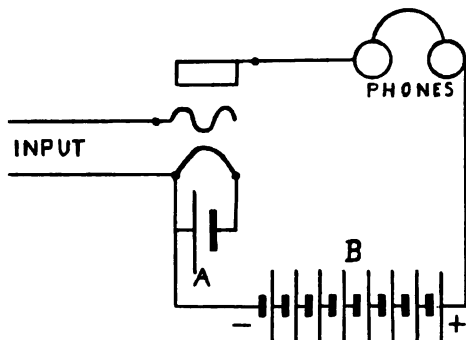


Fig. 2. Typical arrangement of electron tube and circuits commonly used for amplifying currents and detecting them with telephone receivers. A, filament-heating battery; B, plate battery.

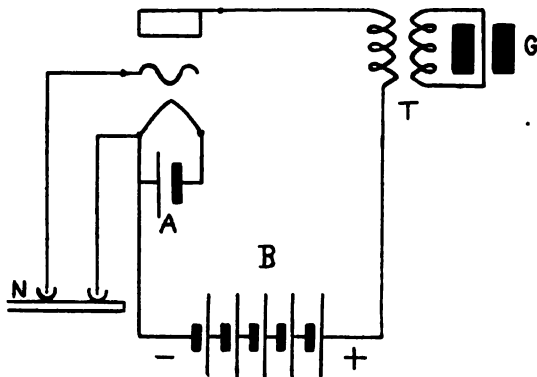


Fig. 3. Transformer method (see text). N, nerve or other tissue giving rise to action current. T, transformer. G, string galvanometer.

fig. 3). The second method was proposed by Mr. S. W. Dean, and is based on the principle of the Wheatstone bridge (fig. 4). It may be designated the bridge method. The third method was proposed by Mr. Sewall Cabot. It consists in protecting the string galvanometer from the direct current by placing it in series with a condenser, and

shunting the plate current by the string and condenser through a resistance of the same order of magnitude as that of the tube (fig. 5).<sup>2</sup> Whittemore (3) has described a scheme for measuring with a string galvanometer radio signals rectified by an electron tube. This procedure, though intended for a wholly different purpose from ours, has much in

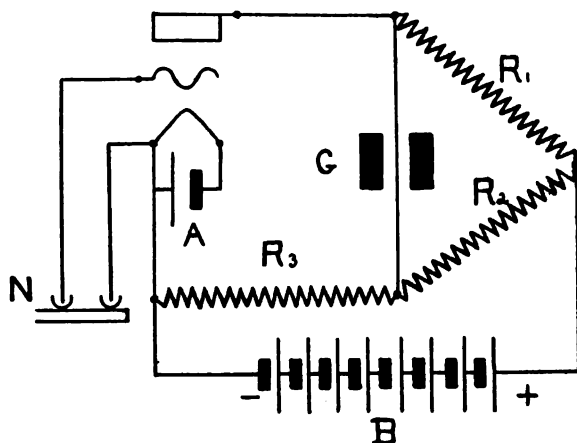


Fig. 4. Bridge method (see text).

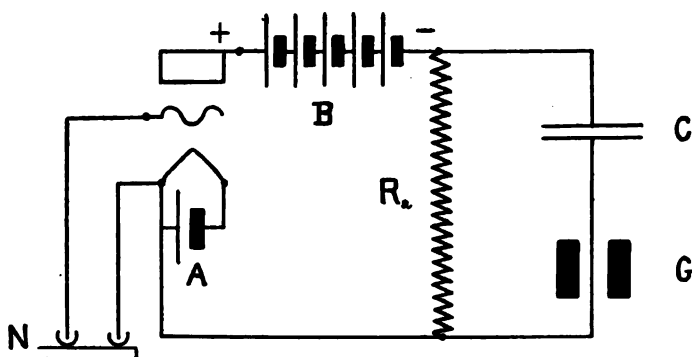


Fig. 5. Condenser method.  $C$ , condenser.  $R_1$ , by-pass resistance (see text).

<sup>2</sup> This method was first tried with the plate battery between the filament and the galvanometer, etc. At the suggestion of Prof. G. W. Pierce, the battery was shifted to the location shown, next to the plate. It makes no difference in the amplification, but it is better practice to have the string on the low potential side of the circuit, and makes it possible to keep the magnet core of the galvanometer grounded.

common with both the bridge method and the condenser method here described.

The transformer method offers the objection that what is recorded is the *rate of change* of the difference of potential to be amplified; a sustained e. m. f. would give no evidence of its presence by this method, except at its beginning and ending. However, as the action current of a nerve-trunk following a single stimulus is of very rapid onset and brief duration,—so brief that no string has time to attain its full excursion before the maximum e. m. f. is over (4, p. 145)—there might be some use in obtaining records that would show a rate of change. It might serve for the comparison of the magnitudes of successive responses in nerve under conditions insuring constancy of time relations. The method seemed at least worth a trial.

The bridge method consists in causing the current from the high voltage (plate) battery to pass through a split circuit, one branch consisting of  $R_1$  and the tube in series, the other consisting of  $R_2$  and  $R_3$  in series (fig. 4). The string connects the two circuits at their middle points. The best results would be obtained with  $R_1$ ,  $R_2$  and  $R_3$  all approximately equal to the resistance of the tube,  $R_b$  (plate-to-filament), and in any case the proportion  $\frac{R_2}{R_3} = \frac{R_1}{R_b}$  must hold.

This method depends on the balance in the bridge, i.e., the equalizing of potential at the two ends of the string. Starting with such a balance, a change in the input difference of potential (between filament and grid) would, by causing a change in the resistance of the tube (plate-to-filament), unbalance the system and create a difference of potential between the ends of the string.

The chief advantage of this method is that changes in the input difference of potential are recorded without distortion; that is, a constant e. m. f. is shown as such in the record.

The chief drawback lies in the difficulty of obtaining a perfect balance, and of maintaining it through an experiment. Another objection is that the resistance of any string commonly in use is so low, compared with those in the bridge, that it approximates a short-circuit and tends to reduce the difference of potential set up between the two points it connects. This tendency will reduce the resulting amplification.

The condenser method, suggested by Cabot, obviates both of these difficulties. There being no current permitted to pass as long as the tube resistance is steady, on account of the interposed condenser, the

problem of balancing does not arise. There is also no such reducing tendency as was mentioned above. The principle of the method is in the main the same. It depends on the fact that if a constant voltage drop occurs through two resistances in series, when one of these changes (the other remaining constant), the voltage drop through it must change; consequently, a change of potential occurs at the end of the string connected to the junctional point where the two resistances are connected.

At first sight it would appear that the condenser would lay this method open to the same objection that applies to the transformer method, that it would distort the record, showing only a rate of change in the input, and failing to record a sustained e. m. f. as such. This objection can readily be overcome for practical purposes by making the condenser very large, so that it provides a virtually constant reservoir of energy throughout the time that an action current lasts. We are chiefly concerned with the action currents of nerves, and these are of brief duration, most of the effect being over in less than 0.01 second; action currents of cardiac or skeletal muscle rarely require amplification. The resistance through which the condenser has to discharge itself with this arrangement under optimum conditions amounts to about 80,000 ohms. Through such a resistance a condenser of 25 mf. requires considerably over 2 seconds for approximately complete discharge; in 0.01 second it would lose 0.5 per cent of its charge. In short, for recording an electrical disturbance lasting only 0.01 second, the method would not introduce any appreciable distortion with a condenser of this order of magnitude. In the case of action currents of skeletal muscle, although their duration is much greater than those of nerve, the distortion is still insignificant. When currents of longer duration are studied, it is usually possible to obtain all the amplification that is needed by slacking the tension on the string. Electron tube amplification, therefore, has its chief use in studying brief and rapidly changing electrical disturbances which a slack string will not follow satisfactorily.

#### APPARATUS

The electron tubes used in this research were generously loaned us by the Western Electric Company. On the recommendation of Dr. H. B. Arnold "D-tubes" and "L-tubes" were tried, two of each being furnished, with a socket to fit them.

A transformer was also loaned by the Western Electric Company to enable us to try the first-mentioned method. It was an autotransformer with a closed iron core. The windings were in four sections, two of 12.5 ohms each and two of 62.5 ohms each, making a total resistance of 150 ohms. The condenser used in the third method was made of the common Western Electric Company paper condensers of 1 microfarad each, connected in parallel. In nearly all of the experiments 15 were used, giving thus a capacity of 15 mf.

The current for heating the filament was supplied by an Edison storage battery of five type B-2 cells, having a capacity of 40 ampere-hours. This is conventionally known as the "A-battery." The current was regulated by a small circular rheostat on a porcelain base, such as is commonly used for this purpose. The battery which supplied the plate current (conventionally known as the B-battery) consisted during the experimental stage of the work chiefly of the small dry cells used for flash-lights, connected in series. This was supplemented by a 24-cell lead storage battery loaned by the Cruft Laboratory of the Harvard Physics Department. When the apparatus was finally arranged as a permanent installation a B-battery of lead storage cells was made in the following way:

A lead strip 1 inch wide by  $\frac{1}{16}$  inch thick was passed through a press at the Jefferson Physical Laboratory, thus impressing on it a pattern which increased the surface area; then the strip was cut into suitable lengths. A disc-shaped mould was prepared with two slits through which the lead strips fitted, and with a wire nail protruding upward through the bottom. Two lead strips were passed through the slits to the proper distance and a string tied round them within the mould, then a short piece of glass tubing was put over the wire nail. Then a mixture of paraffin, beeswax and rosin was poured into the mould and allowed to harden, the string serving to strengthen the wax, and the glass tube providing a vent. Small glass jars about 2 inches by 6 were filled with sulphuric acid, and the wax discs with the lead strips protruding downward were fitted into the necks and the openings sealed with hot wax, all but the vents. A wooden separator was placed between the plates of each cell. One hundred and thirty-five of these cells were made and set up in three trays. Each tray had 45 cells connected in series. In order to form the plates the three trays were connected in parallel and charged from the 110-volt mains first in one direction and then in the other. The charging current proved to be so small that to hasten the process storage batteries were con-

nected in series with the 110-volt mains, giving a total charging voltage of 130. When the plates were formed the batteries were connected with a group of three switches so wired that by throwing them all one way the three groups of cells were arranged in series and connected with the system, and by throwing them all the other way the three groups were arranged in parallel and connected with wires whereby they could be charged from the 110-volt power mains. This switching arrangement is included in the general wiring plan shown in figure 17.

The galvanometer used was an Einthoven String Galvanometer furnished by the Cambridge Scientific Instrument Company of Cambridge, England; the same that has been used in this laboratory for several years. In most of the experiments a comparatively coarse platinum string was used, having a diameter of 5 micra and a resistance of 910 ohms (designated string *B*); in some experiments we used a gilded quartz string, furnished by Hindle, of 2.5 micra diameter and 20,000 ohms resistance (string *G*); in the last experiments we used a similar Hindle string of 12,000 ohms.

In order to protect the string during the establishment of the high voltage circuit, and in preliminary tests, a shunt was connected across the terminals of the double-pole switch used to connect the string with all other circuits. For this purpose we used a rheostat of the same type as that used to control the filament current.

For projection we used an arc lamp furnished by A. T. Thompson. As usual, a cylindrical lens was used to focus the beam of light in a horizontal line across the film.

For recording we at first attempted to use a camera in which the film was moved by means of a Sandstrom electric kymograph. This was abandoned for a reason which will be discussed later, and all experiments were performed with a newly constructed camera, used for the first time in this research. Since this camera has proved exceedingly useful and convenient for a wide range of physiological work, and has not yet been described, it is thought worth while to include here a brief description, especially since one feature of it proved essential to success under the conditions met with in these experiments. The camera was designed to make records on standard perforated moving picture film, the motion of which is regulated by a sprocket such as is used in moving picture cameras and projectors. The sprocket is so placed behind the illuminated slit that it presents the surface of the film at the focal plane of a cylindrical lens set in an adjustable holder in the path of the beam of light. It is mounted

on a shaft provided with a conical clutch whereby it may be rapidly started and stopped, the clutch connecting it with a friction drive which is run by an electric motor. The motor is a 12-volt D. C. machine intended for an automobile self-starter, and is completely encased in metal; it runs at 460 r. p. m. This motor turns the disc of the friction drive, which is of cast iron and has a diameter of 32 cm. A spring on the shaft holds this disc firmly in contact with a wheel with a leather rim 18.5 cm. in diameter mounted on the horizontal shaft which

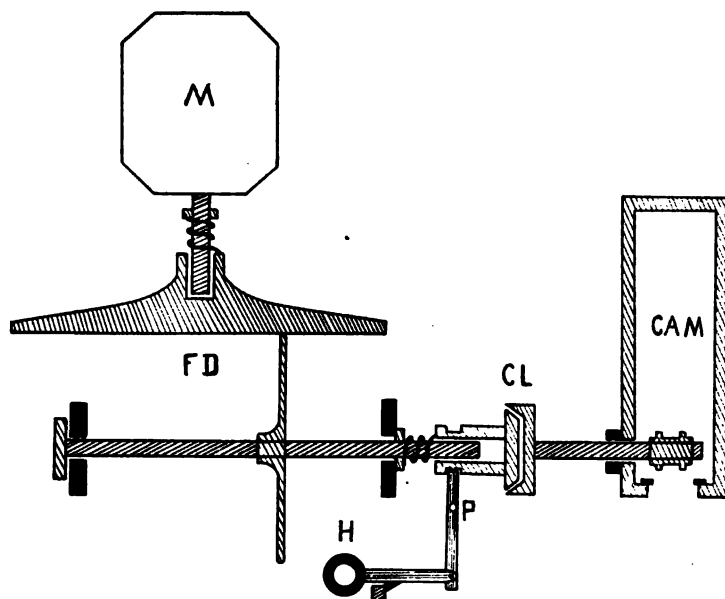


Fig. 6. Horizontal section of photographic recording apparatus at level of optical axis. *M*, motor. *FD*, friction drive. *CL*, clutch. *H*, handle for operating clutch, showing spring, lever pivoted at *P*, and catch. *Cam*, camera.

carries the clutch, and is perpendicular to the motor shaft. The wheel may be moved along the shaft from the center to the rim of the disc, and secured in any desired position with a set-screw, thus providing a wide range of gear ratios (fig. 6). In this way any desired speed of film is obtained, from a minimum of about 7 cm. per second to a maximum of 48 cm. per second. Still slower speeds could be had by putting a resistance in series with the armature of the motor. The film, which comes in 200-foot rolls, is placed in a light-proof magazine of a type used in some moving picture cameras: in this magazine it may be in-



serted in the camera in full daylight. The end of the film is led around the sprocket and into a receiving magazine so arranged that the spool on which it is received is coupled to the shaft of a spring clock-work device which serves to wind up the film as fast as it leaves the sprocket (fig. 7). The lever which operates the clutch also moves a screen which cuts off the beam of light when the film is stopped. This prevents fogging of the film during short intervals between observations. This fogging spoils the film for about an inch on each side of the point behind the slit; on a series of brief observations at a slow speed of film the percentage of waste would be considerable without this screen. The current which drives the motor is furnished by a battery of Gould storage cells of large capacity, which maintains practically constant speed.

This camera has several advantages over one previously described (4, p. 127) and used hitherto in recording with this galvanometer. It carries 200 feet of film at one loading, as compared with 50 feet, which was practically the maximum capacity of the old camera; and it can be loaded by transferring the magazine to the dark room without moving the camera, which is large and heavy. The old camera had only certain fixed speeds depending on the gear ratios of the Sandstrom kymograph. The friction drive makes it possible to select any speed

within the limits mentioned. The most important advantage is the unfailing uniformity of speed imparted to the film by the sprocket engaging in the perforations. It was the great difficulty in obtaining uniform motion with a film pressed against a revolving cylinder by rubber rollers, that led to the abandonment of the old camera. In this particular research an unexpected and indispensable advantage was found in the new camera; this has already been alluded to, and will be discussed later. The chief limitation of the new camera lies in the narrowness of the film (35 mm.), this being the maximum width made with perforations. This difficulty can be overcome by moving the camera nearer to the galvanometer and thus decreasing the magnification. Sharper definition of the image results, and greater contrast in the film

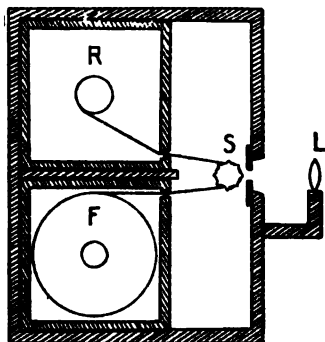


Fig. 7. Vertical section of camera. *F*, feeding spool in magazine. *R*, receiving magazine. *S*, sprocket. *L*, cylindrical lens.

at high speeds. If it is desired to reproduce the record on a larger scale it may easily be enlarged, and the increased definition and contrast will facilitate the process.

#### ELEMENTARY EXPERIMENTS

For comparison of the results obtained with and without the electron tube, we used in general an artificial source of current to be amplified, instead of the action current of a nerve or muscle. This had the great advantage of saving time, for any desired electromotive force could be drawn at a moment's notice without the labor of dissecting a nerve, placing it in a moist chamber and connecting it with

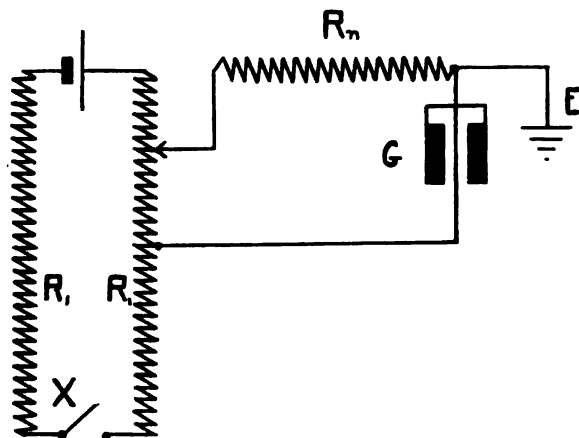


Fig. 8. Standard wiring diagram (simplified).  $R_1$ , resistance in primary circuit.  $X$ , key in primary circuit.  $R_n$ , resistance representing resistance of tissue in series with galvanometer,  $G$ .  $E$ , connection to earth.

non-polarizable electrodes. Even greater advantages were the dependable uniformity of the source of current and the ease with which its quantity and duration could be regulated, conditions impossible of fulfillment with living tissues. The source of current was an Edison cell connected with a resistance of 640 ohms,  $R_1$ , in series with a slide-wire 1 meter long and having a resistance of 4.8 ohms. This established a potential drop of 0.0001 volt per centimeter along the slide-wire, and made it convenient to select readily the desired e. m. f. From one end of the slide-wire and from the sliding contact, wires were led either to the string galvanometer or to the filament and grid of the electron

tube; this may be termed the input circuit. In order to simulate as far as possible the electrical conditions which obtain in recording the action currents of living tissues, a resistance representing the resistance of the tissue was introduced in the input circuit in the path from the sliding contact to the galvanometer or the grid of the electron tube, as the case might be. In the great majority of experiments this resistance, termed  $R_n$ , was 10,000 ohms, this value being selected as a convenient one which is about the lower limit of resistances found in experiments on nerve, and about the upper limit in the case of muscle preparations. The key which was used to make and break the current was placed in series with the Edison cell and the resistance  $R_1$ , the

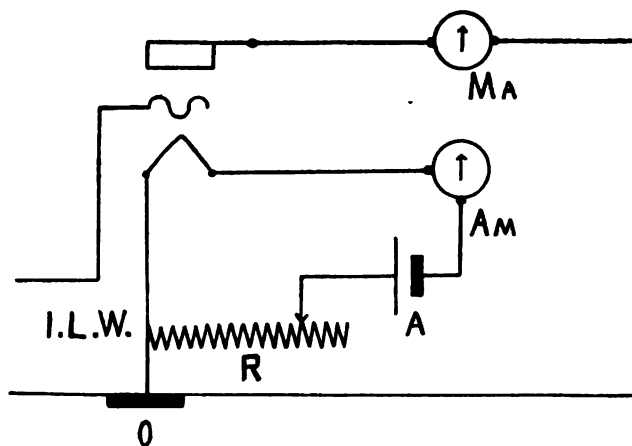


Fig. 9. Wiring diagram of electron tube panel. *I L W*, input lead wires. *O*, brass bar at point of zero potential. *A*, filament-heating battery. *R*, rheostat for controlling filament current. *AM*, ammeter in filament circuit. *MA*, milliammeter for plate current.

input circuit from slide-wire to galvanometer (or to electron tube) remaining closed. The circuit diagram of the standard wiring arrangement reduced to its simplest terms, is shown in figure 8 (cf. 4, fig. 1). This arrangement was the standard with which all the various electron tube methods were compared.

The socket provided for the tubes was mounted on a board, together with the rheostat for controlling the filament current, an ammeter for measuring the same, and a milliammeter for measuring the plate current. The board was permanently wired in accordance with the diagram in figure 9. To a brass bar mounted on the board were soldered

wires, leading to the filament and its rheostat, to the negative pole of the B-battery (plate circuit), and another wire for connection with the source of current to be amplified. This last and a wire soldered to the grid terminal of the socket, may be termed the input lead wires. The brass bar, being at the junctional point of the various circuits, a point normally put to earth and thus maintained at zero potential, may be termed the zero point.

First a series of measurements was made to determine the characteristics of the tubes; that is, to determine the plate current values obtained with various values of filament current (determining cathode temperature), plate potential and grid potential. Doctor Arnold had advised us that the D-tube should be operated at a plate potential in the neighborhood of 200 volts. With this voltage we found that the plate current increased rapidly with the filament temperature till the filament-heating current attained a value of about 1.10 amp.; beyond this point the increase was much less rapid. Van der Bijl (1, p. 175) shows that the device should operate with a filament temperature sufficient to maintain more available electrons than the space charge will permit to travel to the plate; that is, in the present case, with a filament current of 1.10 amp. or more. Most of our experiments were performed with a filament current of 1.20 amp. Figure 10 shows the characteristics of one of the D-tubes<sup>3</sup> with the filament current maintained at 1.20 amp. In figure 10 A the values of plate current,  $i_b$ , are plotted against plate voltage,  $E_b$ , for different values of grid potential,  $E_c$ . In figure 10 B, the plate current is plotted against grid potential at different plate voltages. It should be noted that very different scales are used for grid and plate potentials. Were the curves plotted on the same scale, those in figure 10 B would be much steeper than those in figure 10 A. To make the grid potential zero, (with respect to filament) we connected the grid with the filament through a 10,000 ohm resistance. For various positive and negative values of grid potential we connected the grid with the filament through one or more dry cells in series. From the shape of the curves in figure 10 A it will be seen that the resistance of the tube does not remain constant when filament temperature and grid potential are kept constant, but decreases as the plate voltage is increased; it is a variable dependent on the values of all three determining factors, filament temperature, plate and grid potentials. This is a fact of fundamental importance in dealing with the electron tube, as will be seen later.

<sup>3</sup> The two D-tubes agreed closely in their characteristics.

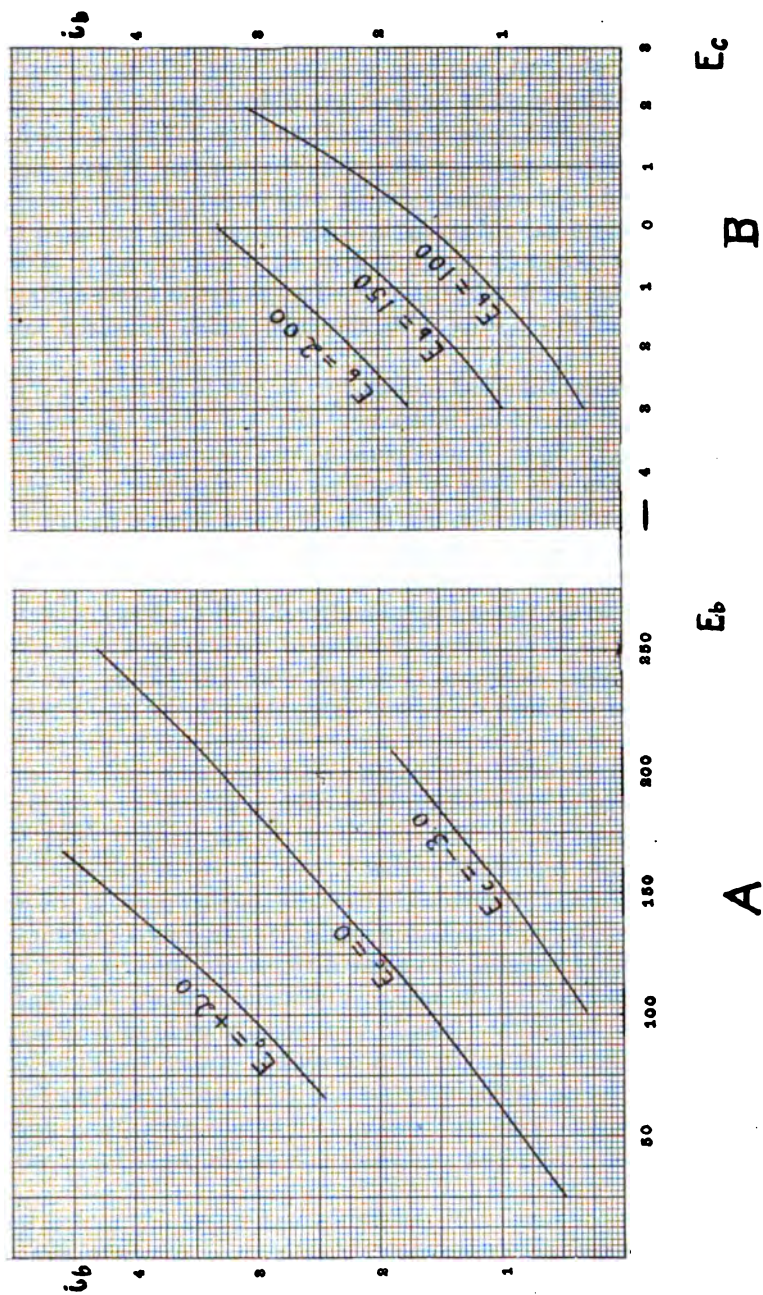


Fig. 10. Characteristic curves of D-tube with filament current of 1.20 amp. (see text). Ordinates, milliamperes. Abscissae, volta.

## THE CONDENSER METHOD

The first method tried was the condenser method suggested by Cabot (fig. 5). This method showed promising results at the outset. Both D-tube and L-tube were tried, and the D-tube showed in the preliminary test so much greater amplification that it was thereafter adopted and used regularly in all subsequent experiments. It is possible that more careful study and selection of the values of the various constants would have resulted in some improvement of the performance of the L-tube, but it seemed to us better to concentrate on the analysis of the results with the tube which appeared most promising.

The board with the tube and meters, the A- and B-batteries and the condenser were all placed on tables near that on which the cell and resistances shown in figure 8 were permanently installed. The wiring of these resistances was then so modified and supplemented as to make the entire arrangement conform to the basic scheme in figure 5. The detail shown in figure 9 applies to all experiments, but most of it is omitted in the other diagrams for the sake of simplicity. The protective shunt across the string, already referred to, was connected with those terminals of the switch leading to the string, which, when the switch was open, were not connected with the string. In this way the condenser obtained its initial charge through the shunt before this switch was closed. This is an important safeguard to the string. Figure 11 shows contrasted the standard arrangement and the condenser arrangement of the electron tube circuits intended to simulate those of physiological work. The same e. m. f. was tapped from the resistance slide-wire, representing that arising in the tissue, and the same resistance,  $R_n$ , was introduced in each case at that point in the circuit where the resistance of the tissue would be. In some experiments the initial potential of the grid was allowed to remain the same as that of the filament; in that case no cell was introduced at  $E_c$ . In others the grid was given an initial "bias," i.e., a positive or negative potential, by inserting one or more dry cells in series between the slide-wire and grid. After a few preliminary tests with the artificial source of current some practical tests were made with action currents in nerve and muscle. The description of these will be reserved for a later section.

After adjusting the tension of the string a photographic record was made with the standard arrangement, showing the excursion due to

the passage of a current through the galvanometer, the circuit being opened and closed by hand with a key ( $X$  in fig. 11). Sometimes a metal spring key was used, sometimes a key in which a sharp amalgamated copper point made contact with a cup of mercury. After making this control record we rewired the apparatus for the electron tube test without disturbing the adjustment of the string tension. With the string circuit still open and the shunt closed, we turned on the filament current and established the plate current through  $R_2$ . When all adjustments were made we closed the switch leading to the string, and finally opened the protective shunt. The string showed a marked displacement, in some instances disappearing from the field. This indicated a slight leakage of current through the condenser. Measurements showed that when 15 microfarads were used the resistance of

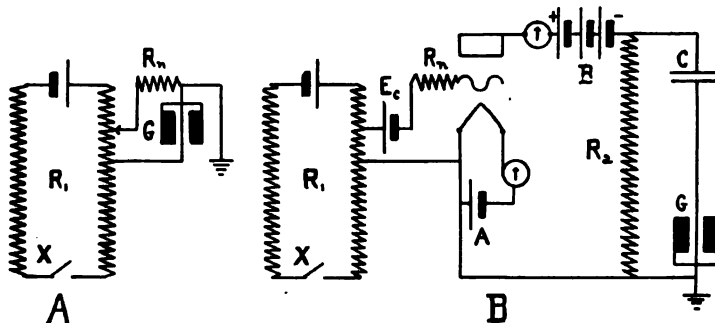


Fig. 11. Comparison of arrangements for experiments on amplification. A, standard arrangement. B, electron tube arrangement, condenser method. Notation as in other figures.

this leak was 32 megohms. It could be avoided by using carefully made mica condensers. A much cheaper way out of the difficulty will be described later. But in most of the experiments we adopted the crude but simple expedient of moving the fibre case of the galvanometer laterally till the string, though deflected by the current, was again in the middle of the field. The input e. m. f. was then applied by making and breaking the circuit at  $X$  and the resulting excursions observed and recorded photographically.

It was at this point that we encountered the difficulty already mentioned in recording with the Santsstrom kymograph camera. As soon as the kymograph motor was started the string began to oscillate violently. The same occurred when we turned on the electromagnetic

tuning fork which shows hundredths of a second by its shadow on the film. The Sandstrom kymograph is run by a 110-volt D. C. motor which works on the regular power line; it has a centrifugal governor which maintains a constant speed by breaking the circuit through a part of the armature windings when the speed exceeds this constant value. The action of this governor is attended by visible sparking at the centrifugal contact. The electromagnetic tuning fork was operated on a split circuit from the power mains with a lamp in series and a resistance in parallel. The tuning fork is kept vibrating by means of an electromagnet, on the principle of an ordinary buzzer.

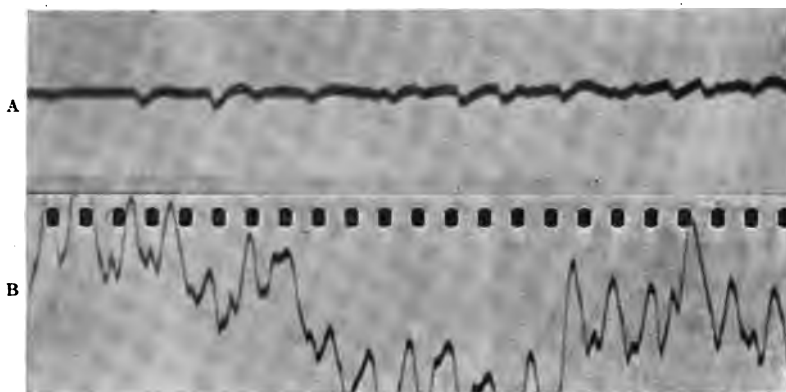


Fig. 12. Oscillations induced by 110-volt kymograph motor in string galvanometer connected with electron tube as in figure 11 B.  $I_A$ , 1.10;  $E$ , 280;  $E_c$ , -1.5;  $R$ , 60,000. A, when motor is first started. B, after centrifugal governor has begun to operate (see text). String B. Tension, 1 cm. excursion =  $2.75 \times 10^{-7}$  amp. Speed of film, 15.6 cm. per second. In this and all other photographic records the magnification is 300 diameters. In all records made with electron tubes the following symbols are used:— $I_A$ , filament current;  $E$ , plate battery voltage;  $E_b$ , plate potential;  $E_c$ , initial grid potential;  $R$ , by-pass resistance (hitherto designated  $R_2$ ). In all records made with condenser method condenser capacity was 15 mf.

Both the kymograph and the tuning fork had two features in common, connection with the 110-volt power circuit, and a circuit-breaking contact at which sparking occurred.

In the case of the kymograph motor, some oscillations were visible as soon as the motor was turned on, but they enormously increased in amplitude as soon as the centrifugal governor began to operate with its attendant sparking. (See fig. 12.) It was clearly impossible to



record any experiment with a camera depending on this kymograph motor, at least without elaborate shielding. Such shielding as was mechanically feasible failed to stop the oscillations. We therefore substituted for it the new camera described above, which had just been completed for general use with the string galvanometer. It was then found that the friction of the leather rim of the wheel with the iron disc in the friction drive produced static electricity to such an extent that sparks jumped occasionally from the metal part of the wheel to the disc. The running of this motor caused no oscillation of the string when connected with the electron tube arrangement, except when one of these sparks was seen to jump; then the string made a quick excursion. This difficulty was remedied by grounding both the disc and the wheel of the friction drive, using a copper brush to make continuous contact with the revolving disc. After this change it was found possible to obtain perfectly smooth records with this camera, there being no oscillations in the string induced by the running of the motor. It should be noted that this was a 12-volt motor, run from a storage battery in the room, having no connection with the power mains, and that the motor was completely encased in metal.

The oscillations induced by the tuning fork were found to be considerably reduced in amplitude when, later on, the electron tube and accessories were permanently installed on a lower shelf on the same table with the resistance and switches connected with the galvanometer. The oscillations were still further reduced somewhat when the tuning fork was operated by a 6-volt battery instead of being connected with the power mains (fig. 13). The persistence of oscillations even under these conditions showed the powerful effect of sparking in inducing such disturbances.

The cause of the disturbance was evidently some form of electromagnetic induction, probably due to high frequency currents resulting from sparking, which by their action on the grid circuit, caused disturbances in the plate circuit, by virtue of the rectifying action of the tube. It was feared that a similar disturbance would result from the use of break shocks of an ordinary induction coil for stimulating the tissues to be studied, for the break of the primary current is necessarily attended by a spark. An inductorium was set up in the position commonly used for stimulating, and as large a primary current as is ever used with this coil was broken while the galvanometer was connected with the electron tube as indicated. No excursion of the string resulted unless the hand of the experimenter touched the

primary circuit. With such contact established, the making and breaking of the primary circuit caused excursions of the string. Evidently such sparking as occurred in the inductorium as used in the laboratory, did not suffice to produce any excursions of the string which might confuse an experiment. But it was necessary to insure insulation of the primary circuit from the experimenter, to avoid confusing excursions of the string.

In subsequent experiments the tuning fork was not used, and the constancy of the speed of the motor was relied upon for measurements

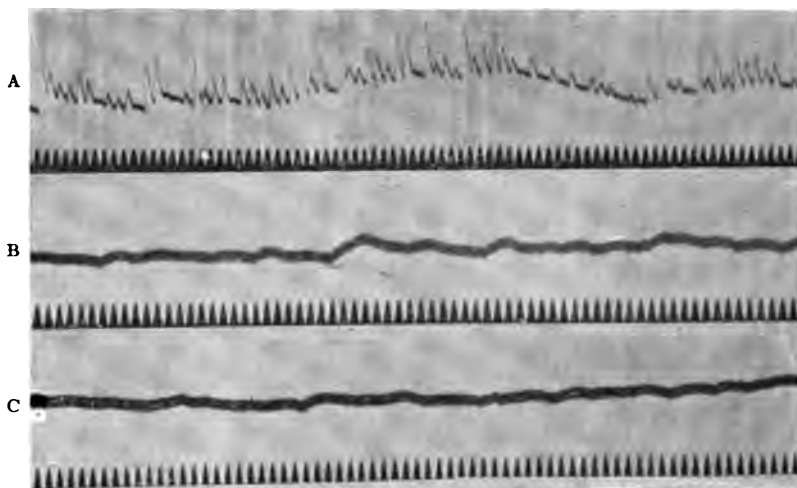


Fig. 13. Oscillations induced by electromagnetic tuning fork. A, string *H*; tension, 1 cm. =  $5.9 \times 10^{-7}$ .  $I_A$ , 1.10;  $E$ , 300;  $E_c$ , -1.5;  $R$ , 50,000. B and C string *B*; tension, 1 cm. =  $2.75 \times 10^{-7}$  amp. (as in fig. 12).  $I_A$ , 1.10;  $E$ , 280;  $E_c$ , -1.5;  $R$ , 60,000. In A and B the tuning fork was operated on the 110-volt power circuit; in C it was operated by a 6-volt battery. Time indicated by shadow of tuning fork; each complete vibration = 0.01 second.

of time. This constancy was found experimentally to be quite adequate for our purposes. A short strip of film with the tuning fork operating was exposed before the experiment, and another one afterwards, by way of speed control.

Referring to figures 5 and 11 B, it will be noted that part of the voltage of the B-battery is used up in the resistance  $R_2$ , leaving so much less for the tube. In other words, the plate-to-filament resistance of the tube being in series with the resistance  $R_2$ , part of the potential

drop in the circuit occurs in the latter, and only the remainder within the tube. On this account it is necessary to provide a battery of higher voltage than that which would be required to operate the tube without such a resistance in series. It is also necessary to determine, among other things, the value of  $R_2$  which will give the best results.

The question of values of the various resistances and voltages involved will be discussed later in detail. For the present it will suffice to say that the condenser method was tried with various values of plate battery voltage from 113 to 400, and with values of  $R_2$  varying from 10,000 ohms to 120,000 ohms. The results of fairly typical experiments are shown in figure 14. A shows the amplification with the low resistance string; B shows the amplification under comparable conditions with the high resistance string.

Marked amplification in the excursions of the string is obvious in both cases. It is also noticeable that with the electron tube arrangement the decline of the current through the string due to the gradual discharge of the condenser is very small in as brief a time as that shown in the figure; in the case of figure 10 B, amounting to about 0.08 second. From this it follows that for disturbances as brief as the action currents of nerve and skeletal muscle, the distortion from this cause is negligible, as was predicted. It is also noticeable that the amplification is considerably greater in the case of the high resistance string than in that of the low resistance string. This is due to the fact that a low resistance string is relatively

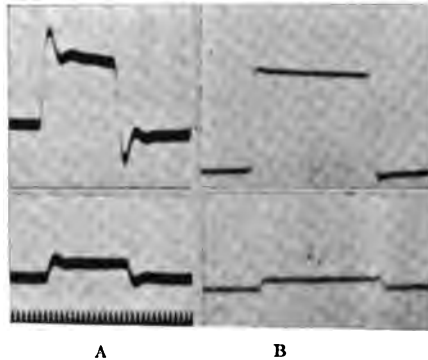


Fig. 14. Amplification by electron tube, condenser method. Upper row, electron tube arrangement; lower row, standard arrangement. In all records of test current with electron tube  $\Delta E_c$  is change in grid potential used to make record. Other notation as explained in legend to figure 12.

A, string B; tension, 1 cm. =  $2.75 \times 10^{-7}$  amp. Upper row:— $I_A$ , 1.25;  $E$ , 240;  $E_b$ , 170;  $E_c$ , — 3.0;  $R$ , 90,000;  $\Delta E_c$ , 0.0006;  $R_n$ , 10,000. Lower row (standard)  $\frac{0.0006}{S + 10,000}$  (i.e., 10,000 ohms in series with string). Tuning fork shows time in 0.01 sec. intervals.

B, string G; tension, 1 cm. =  $5.0 \times 10^{-7}$ . Upper row:— $I_A$ , 1.25;  $E$ , 238;  $E_b$ , 170 (approx.);  $E_c$ , — 3.0;  $R$ , 90,000;  $\Delta E_c$ , 0.0020;  $R_n$ , 10,000. Lower row,  $\frac{0.0020}{S + 10,000}$ . Speed of film, 20 cm. per second.

placed at a disadvantage as compared with a high resistance string when connected in series with the resistances involved in the electron tube circuit, which are very much greater than the resistance of either string. A large external resistance makes less difference to the resulting current if the string resistance is large than if it is small.

We found that when the initial grid potential was zero,—that is, when no cell was introduced to give it a negative bias, the grid circuit absorbed a certain amount of current. This was shown by the fact that the excursions were made considerably smaller when a 10,000 ohm resistance,  $R_n$ , was introduced into the grid circuit, than when this resistance was left out. In one experiment this reduction amounted to 32 per cent. When one or more dry cells were inserted in the grid circuit, thus making the grid potential negative to the extent of 1.5 volts or more,<sup>4</sup> the grid circuit took no current. This was shown by the fact that the insertion of as much as 130,000 ohms made no difference whatever in the magnitude of excursion. In this sense the apparatus assumed the function of an electrometer. The consequence is that the electron tube will amplify the electrical disturbance of a tissue of high resistance, very much more than it will that of a tissue of low resistance and the relative gain is greater for a low resistance string than it is for a high resistance string. For instance, in the case of our low resistance string,  $B$ , with only 910 ohms resistance, there is no amplification at all in measuring an electrical disturbance set up in a circuit of negligibly small resistance; there is on the contrary a reduction. In other words, when a given difference of potential is applied to the terminals, this low resistance string will give a larger excursion than it will if connected with the electron tube when the same difference of potential is applied to the grid circuit. The conclusion to be drawn from this is that in studying a tissue of low resistance like skeletal muscle, with a low resistance string little or nothing would be gained by use of the electron tube, but that in studying a high resistance tissue such as a nerve trunk very great amplification is to be obtained. This point will be taken up quantitatively later on.

#### THE TRANSFORMER METHOD

In testing the transformer method it was still necessary to protect the string by placing a condenser in series with it, since the transformer at our disposal was an autotransformer, that is, the windings

<sup>4</sup> A third D-tube used later in some of the final experiments required 3 volts negative grid potential to bring about this result.

were all connected in series, with a number of taps available for connecting the circuits. Thus it was impossible to insulate the secondary circuit from the primary, and consequently without a condenser the string would have been subjected to a large direct current from the B-battery. On the advice of Mr. Colpitts, the coil was connected as a step-down transformer; that is, all four windings were included in the primary, or plate circuit, and only one of the two larger windings was included in the secondary or galvanometer circuit. The reverse arrangement was also tried.

We used values of filament current and plate potential which had given good amplification with the condenser method. It was evident at once that the transformer method was inferior in every respect. Figure 15 shows a comparison of the galvanometric excursions obtained with the standard arrangement, with the condenser method and with the transformer method. In this experiment the essential electron tube values were the same with the transformer method as with the condenser. It should be noted in this connection that the entire plate battery voltage is applied to the plate, none of it being expended in the transformer, whose resistance is negligible compared with that of the tube. The record shows that not only does the transformer method record only the rate of change of the electrical disturbance, but that the amplification of the initial excursion is very much less than that obtained with the condenser method. Reversing the transformer produced a record practically identical with that shown in the figure, but a trifle smaller still. No further experiments were tried with this method.

#### THE BRIDGE METHOD

In one experiment we tried the bridge method suggested by Dean (see fig. 4). It proved a difficult matter to balance the four resistances

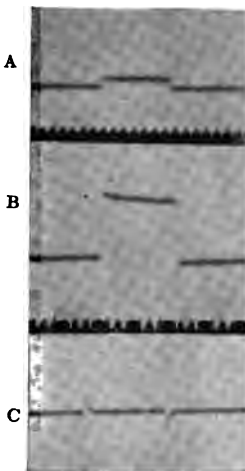


Fig. 15. Comparison of transformer and condenser methods. String G.

A, control observation with standard arrangement. Tension, 1 cm. =  $6.0 \times 10^{-7}$  amp.  $\frac{0.0020}{S + 10,000}$ .

B, condenser method. Tension, 1 cm. =  $6.0 \times 10^{-7}$  amp.  $I_A$ , 1.25;  $E$ , 306;  $E_b$ , 163;  $E_c$ , 0;  $R$ , 50,000;  $\Delta E_c$ , 0.0020;  $R_n$ , 10,000. Tuning fork struck by hand just before observation.

C, transformer method. Tension, 1 cm. =  $5.0 \times 10^{-7}$  amp.  $I_A$ , 1.25;  $E$ , (same as  $E_b$ ) 163;  $E_c$ , 0;  $\Delta E_c$ , 0.0020;  $R_n$ , 10,000.

with sufficient accuracy to keep the string of the galvanometer in the field. And after a balance had been found with difficulty, it was also difficult to maintain it, for the string kept travelling slowly sidewise as a result either of the running down of the A-battery or the B-battery, or of the change of temperature in the tube.

A few records were taken with this method, and as was expected, they showed no great difference from those taken with the condenser method. The chief difference is that there is no falling off during the

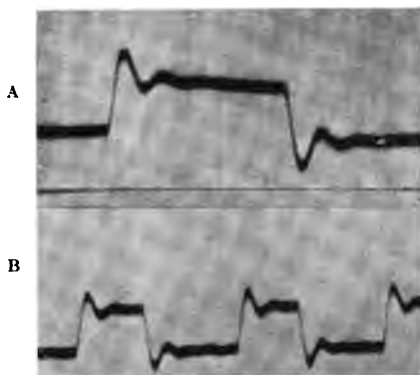


Fig. 16. Comparison of bridge and condenser methods, showing in bridge method absence of decline resulting in condenser method from discharge. String B.

A, condenser method. Tension, 1 cm. =  $5.50 \times 10^{-7}$  amp.  $I_A$ , 1.25;  $E$ , 250;  $E_c$ , 0;  $R$ , 50,000;  $\Delta E_c$ , 0.0010;  $R_n$ , 10,000. Speed of film, 19.5 cm. per second.

B, bridge method. Tension, 1 cm. =  $2.75 \times 10^{-7}$  amp.  $I_A$ , 1.18;  $E$ , 223;  $R_1$ , 40,000;  $R_2$ , 30,000;  $R_3$ , 70,700;  $E_c$ , 0;  $\Delta E_c$ , 0.0010;  $R_n$ , 10,000. Speed of film 10.7 cm. per second.

record of a continued current, such as occurs in the condenser method. On the other hand the gradual shift of base line due to the disturbance of balance mentioned above, though not as rapid as that due to the discharge of the condenser in the other method, is more troublesome in practice. The amplification was also appreciably less with this method than with the condenser. This is probably due to the fact that the experiment was made with the low resistance string whose resistance was so low that it approximated a short-circuit of the bridge. Figure 16 shows records made with the condenser method and the bridge method under approximately comparable conditions, excepting speed of film.

These experiments led us to the conclusion that the condenser method was by far the most satisfactory. We proceeded to install a permanent wiring plan whereby we

could rapidly shift from the standard arrangement to the electron tube arrangement, wired in accordance with the condenser method, by merely throwing a few switches. This served a twofold purpose. It enabled us to make rapid comparisons between the excursions obtained with the standard arrangement and those obtained with the electron tube, thereby reducing to a minimum the error introduced by change

of tension of the string. It also rendered the method permanently available at a moment's notice for the amplification of any action currents found, in the course of a physiological experiment, to be too small to record with the string galvanometer unaided. This wiring plan is shown in figure 17. In the compactness obtained by installing

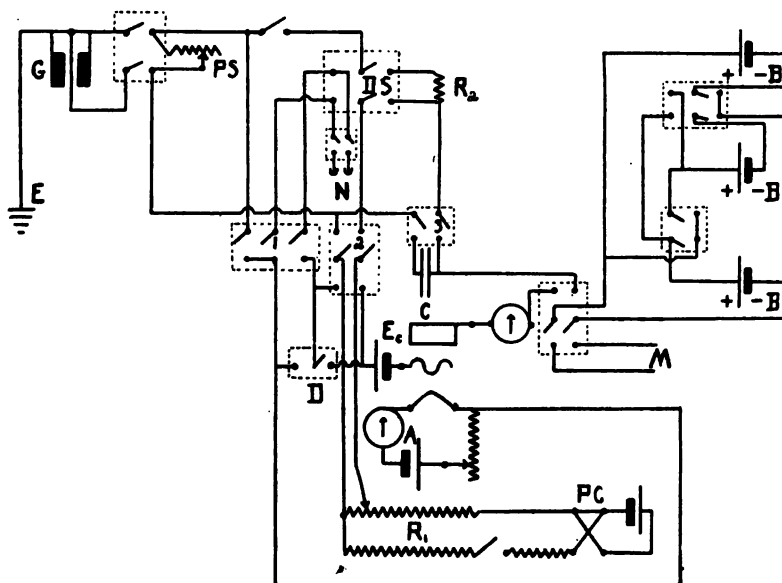


Fig. 17. Complete wiring diagram showing switches for rapid shift from connection of galvanometer directly with tissue or artificial source of current to connection with electron tube. *G*, galvanometer (core grounded). *E*, connection to earth. *PS*, protective shunt. *DS*, switch for connecting galvanometer with tissue or substitution resistance in standard arrangement; in electron tube arrangement this switch is always thrown to right. *R<sub>2</sub>*, in standard arrangement, substitution resistance; in electron tube arrangement, by-pass resistance. *N*, non-polarizable electrodes for connection with tissue. *C*, condenser in series with string. *A*, filament-heating battery. *B*, plate battery in three sections connected with switches for charging them in parallel and discharging them in series. *M*, wires for connection with power mains. *E<sub>0</sub>*, grid bias battery. *PC*, pole-changing switch in source of current used for test or compensation. *D*, single-pole double-throw switch for use in electron tube arrangement. When artificial current is used for test or calibration this switch is thrown to left to short-circuit tissue leads; when recording action currents it is thrown to right and switch *2* is open, thereby disconnecting test current circuit from grid circuit.

In standard arrangement switches *1* and *3* are open, switch *2* is closed upwards. In electron tube arrangement *1* and *3* are closed, *DS* is thrown to right, *2* is closed downward (or open if *D* is to right).

Cf. reference 4, fig. 1.

all the apparatus on shelves of the same table which supported the resistances and switchboard of the old equipment we found the double advantage of convenience and of minimizing the disturbances due to induction in the grid circuit.

#### THE SEARCH FOR MAXIMUM AMPLIFICATION

*General considerations.* Having chosen the condenser method as indicated in figure 5 as that which was most worth developing, it remained to determine if possible how to find those values of the four variables, filament current, grid potential, plate potential, and by-pass

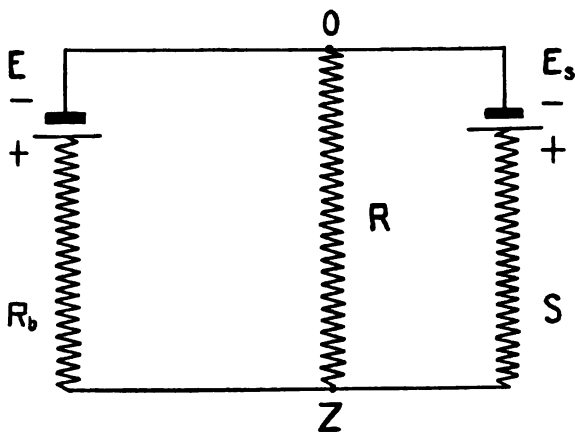


Fig. 18. Simplified schema for analysis of condenser method (see text).  $E$ , plate battery;  $R_b$ , plate-to-filament resistance of tube;  $R$ , by-pass resistance (designated  $R_2$  in figs. 5 and 11);  $E_s$ , battery substituted for condenser;  $S$ , string of galvanometer;  $O$ , junctional point assumed for convenience to be at zero potential in analysis;  $Z$ , junctional point actually put to earth, here assumed to be point of variable potential.

resistance,  $R_2$ , which would yield the maximum possible amplification. Our aim was to find the maximum current in the string which could be made to result from a given change in the difference of potential between grid and filament.

For purposes of analysis we may look on the system as simplified in accordance with the schema shown in figure 18. In this the electron tube and the string are shown simply as resistances, and the condenser is replaced by a battery. In reality a difference of potential is maintained between the plates of the condenser, equal during the resting state, to the voltage drop in  $R$  (representing  $R_2$  in fig. 11), and the



capacity of the condenser is so large that for such brief times as we are dealing with, this difference of potential remains practically constant; for purposes of argument we may look on the capacity as infinite. It acts like a battery whose voltage exactly equals the fall of potential in  $R$  in the resting state, and it is therefore so represented in the analysis.

In practice the zero point which is grounded, and therefore at zero potential, is that point in the system which is connected to the string, the positive end of  $R$ , the filament and one side of the input circuit. But in the following discussion it will simplify matters to assume that the point of zero potential is that which is connected with the negative terminal of the B-battery, the negative end of  $R$  and the negative plate of the condenser ( $O$  in fig. 18). The potentials of all other points in the system are positive with respect to this point, and we may assign to them absolute instead of relative values if we consider the potential of this point fixed at zero. When the difference of potential between grid and filament is raised the resistance of the tube is decreased, and the portion of the total plate battery voltage used up in the tube is decreased. Consequently a rise in potential occurs at that point in the system at which the filament, the positive end of  $R$ , and the string are connected together ( $Z$  in fig. 18). Since the negative plate of the condenser is taken to be fixed at zero potential, the positive plate, (in the present schema, the positive terminal of the battery  $E_s$ ) may be taken to be at a fixed potential  $E_s$ . When the potential of the point  $Z$  is raised, a current will flow through the string from this point to the positive condenser plate. If the potential difference in the grid circuit is lowered the reverse process occurs, everything happening in like manner but in the opposite sense; but to avoid confusion of terms it will be simplest to consider in every case the current resulting from raising the grid potential.

The quantities we must deal with may be designated as follows:

$E$  = total plate battery voltage.

$E_b$  = difference of potential between plate and filament.

$E_c$  = difference of potential between grid and filament, grid potential.

$E_s$  = difference of potential between plates of condenser.

$R_b$  = plate-to-filament resistance of tube.

$R$  = by-pass resistance. (Designated as  $R_2$  in fig. 11.)

$S$  = resistance of string in galvanometer.

$i_b$  = plate-to-filament current within tube.

$i_r$  = current flowing in  $R$ .

$i_s$  = current in string galvanometer.

In the resting state a constant current flows through the tube and the resistance,  $R$ , in series, while no current flows in the string; in other words  $i_b = i_r$ , and  $i_s = 0$ . It is our aim to make the current in the string, resulting from a given change in grid potential as great as possible; in other words, to make  $\frac{di_s}{dE_c}$  a maximum.<sup>5</sup>

The immediate cause of the string current,  $i_s$ , is the rise of potential at the point  $Z$ , or the difference of potential between this point and the positive plate of the condenser; this results directly from the diminution of voltage drop within the tube,  $\Delta E_b$ . This in turn results directly from the change in resistance of the tube. It is therefore our aim to make the change in tube resistance as great as possible without introducing factors which will offset the advantage thus gained. With these considerations in mind we may attempt to find in the tube characteristics a clue to the best method of fulfilling the desired conditions. The problem resolves itself into two phases; (a) how to make a given change of grid potential produce the greatest proportional change in tube resistance; (b) how to make a given change of tube resistance produce the greatest current in the string. We shall consider (b) first, since the result of this consideration will influence our mode of attack upon (a).

Reduced to its simplest terms the fall of potential at the point  $Z$  is that which occurs at the junctional point of two resistances connected in series with a battery of fixed voltage, when one resistance changes and the other does not. This may be illustrated simply in figure 19 ( $R_b$  and  $R$  being transposed for convenience). Let the negative pole of the battery be kept at zero potential, and the positive pole at the potential  $E$ . Then if  $R$  remains fixed, the potential of the junctional point,  $E_b$ , will change when the resistance  $R_b$  changes. It can be shown that for any given ratio between  $R$  and the initial value of  $R_b$ , the change in  $E_b$ , ( $dE_b$ ) depends directly on the *proportional* or percentage change of  $R_b$ ; i.e., on  $\frac{dR_b}{R_b}$  or  $d \log R_b$ . It can also be shown that for a fixed value of  $E$  the change in  $E_b$  for a given proportional change in  $R_b$  is greatest when  $R$  is made equal to  $R_b$ . In other words

<sup>5</sup> For those unfamiliar with this notation it may be explained that  $\Delta$  signifies a definite increment (or decrement if negative) of the quantity designated;  $d$  signifies an infinitesimal increment;  $\frac{dy}{dx}$  signifies the rate at which a variable  $y$  is changing per unit change of a related variable  $x$  at any given time.

$\frac{dE_b}{d \log R_b}$  attains its maximum value  $\frac{E}{4}$  when  $R = R_b$ . When this condition obtains, since  $E_b$  is half of  $E$ , the value  $\frac{E}{4}$  will equal  $\frac{E_b}{2}$ .

If we are free to make the battery voltage,  $E$ , as large as we like, and to make  $R$  correspondingly large in order that the actual plate voltage in the tube ( $E_b$ ) may be kept within proper limits, we can increase the value of  $\frac{dE_b}{d \log R_b}$  (change of potential at  $Z$  for a given proportional change in  $R_b$ ) still more than is possible if  $E$  is fixed and  $R$  is made equal to the initial value of  $R_b$ , as was assumed above.

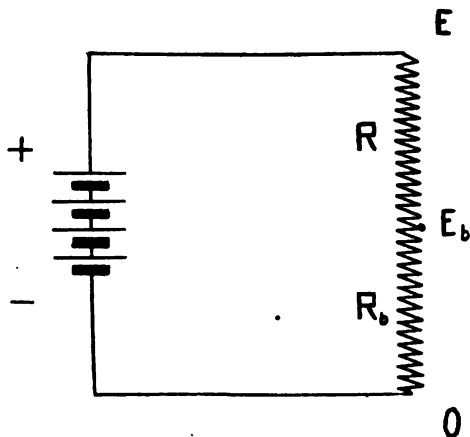


Fig. 19. Schema to illustrate change of potential at junction of resistance in series (see text).

When  $E$  and  $R$  are thus increased,  $\frac{dE_b}{d \log R_b}$  approaches the value  $E_b$  as  $E$  becomes infinitely large. In other words if we operate the tube at its proper plate voltage we can make the desired change of potential almost twice as great by making the battery voltage,  $E$ , very much larger than the plate voltage  $E_b$ , as by making it only twice as large as  $E_b$ . On this account it would appear desirable to make the battery voltage as large as possible and to increase  $R$  correspondingly. In practice the difficulty and danger of having in the laboratory a battery of much more than twice the 200 volts at which the plate of one of these tubes should operate, would be so objectionable as to offset any such small increase in potential as could be had in this way.

From the above we have seen that for our purpose we should seek the maximum proportional change of resistance in the tube for a given change of grid potential. This leads us to study the tube characteristics. Van der Bijl (1, p. 180) gives us the general equation correlating plate-to-filament current with plate and grid potentials,  $i_b = \alpha(\gamma E_b + E_c + \epsilon)^2$ , where  $\alpha$ ,  $\gamma$  and  $\epsilon$  are constants depending on the structure and properties of the tube, and the other symbols have the meanings already assigned to them. Langmuir (5) has given a similar formula chiefly differing from van der Bijl's in that the exponent is  $3/2$  instead of 2. It is generally held that some portions of the characteristic curve obey more nearly the formula of van der Bijl, and other portions the formula of Langmuir. The relations embodied in these formulae are expressed graphically in figure 10. Referring to figure 10 B, showing the relation between plate current and grid potential, and bearing in mind that at a given plate voltage plate current varies inversely with the resistance of the tube, we see that the proportional change of resistance for a given change of grid potential becomes greater and greater as the grid potential becomes more and more negative. It can be shown mathematically that if either van der Bijl's or Langmuir's equation holds, the maximum value of  $\frac{d \log R_b}{dE_c}$  (i. e., proportional change of tube resistance for a given change of grid potential) is obtained at the point in the curve where the current  $i_b$  becomes zero: i. e., where the resistance becomes infinite. If we go too far toward this point on the curve we shall defeat our ultimate aim, for the current in the string  $i_s$  is always less than  $i_b$ , and if this is made excessively small a corresponding limit is placed on  $i_s$ . We must seek that point in the characteristic curve of the tube as determined by cathode temperature and by  $E_b$  and  $E_c$  which, everything considered, will give us the greatest current in the string for a given change in  $E_c$ .

Two ways are open to seek this end, complete mathematical analysis based on the formulae expressing the tube characteristics, and actual experiment with a large variety of systematically chosen values of the independent variables,—filament current, battery voltage, grid potential, and by-pass resistance. Both methods were tried, and the results will be described in turn.

Before leaving the general features of the problem it should be noted that with a given grid potential,  $E_c$ , the current increases more rapidly as the plate voltage,  $E_b$ , is increased than it would if the tube

resistance were fixed. That is, the increase in plate voltage actually lowers the resistance of the tube. The effect of this is to reduce the amplification which would otherwise be obtained. The reason is as follows: Taking our standard case in which the grid potential is raised by a small amount, this change,  $\Delta E_c$ , causes a diminution of the tube resistance,  $R_b$ . But since the plate voltage is not fixed, but depends on the ratio between  $R_b$  and  $R$  in accordance with the relation  $\frac{E_b}{E} = \frac{R_b}{R_b + R}$ , the diminution of  $R_b$  serves to lower  $E_b$ . This in turn, because of the above noted characteristic of the tube, increases the tube resistance. Thus the change in resistance which a change in  $E_c$  tends to produce is in part wiped out by the resultant change in the opposite sense caused by the resulting change in  $E_b$ .

*Mathematical analysis.* In this section we are indebted to Dr. E. L. Chaffee for developing a method of analysis and for many helpful criticisms and suggestions.

We shall continue to assume the condenser replaced by a battery and the point  $O$  (fig. 18) to be maintained at zero potential. The obvious method of searching for the law of maximum amplification would be to find an expression for  $\frac{di_s}{dE_c}$  in terms of all the variables involved (except filament current which we may regard in the mathematical treatment as fixed), and then by differentiating for each variable in turn to find at last the value for each which will give the absolute maximum.

Following this method we may begin with the formulae

$$i_s = i_b - i_r \text{ and } i_r = \frac{E_s + Si_s}{R}$$

derived from Kirchhoff's laws. Combining these with van der Bijl's law for plate current,  $i_b = \alpha(\gamma E_b + E_c)^2$ , (neglecting the quantity  $\epsilon$  which is insignificant), we obtain the formula

$$i_s = \alpha(\gamma E_b + E_c)^2 - \frac{E_s + Si_s}{R},$$

from which by a simple process of algebra we obtain the formula

$$i_s = \frac{R\alpha(\gamma E_b + E_c)^2 - E_s}{R + S}.$$

This expresses the current in the string in terms of the controllable quantities. But when we attempt to differentiate this expression with respect to  $E_s$ , making allowance for the fact that  $E_b$  is a function of  $E_s$ , we encounter insoluble expressions, and the method proves impossible.

An ingenious method has been proposed by Doctor Chaffee whereby a very close approximation to the true value of  $\frac{di_s}{dE_s}$  can be expressed in comparatively simple terms. The method is as follows:—

Referring to figure 18 and applying Kirchhoff's laws we have the following fundamental equations:

$$E_s + i_s S - i_r R = 0, \quad (1)$$

$$i_b = i_r + i_s, \quad (2)$$

$$i_r R = E - E_b. \quad (3)$$

Combining (1) and (2) and multiplying through by  $R$ , we have

$$E_s R + i_b S R - i_r R (S + R) = 0;$$

substituting (3) and rearranging, we have

$$i_b = \frac{(E - E_b)(R + S) - E_s R}{RS}. \quad (4)$$

Differentiating, 
$$\frac{di_b}{dE_b} = - \frac{R + S}{RS}. \quad (5)$$

Changing (2) to read  $i_r = i_b - i_s$ , and combining it with (1), we have,

$$E_s + i_s S - i_b R + i_s R = 0;$$

from which 
$$i_b = \frac{E_s}{R} + \frac{(R + S)}{R} i_s. \quad (6)$$

Differentiating, 
$$\frac{di_b}{di_s} = \frac{R + S}{R}. \quad (7)$$

Thus far the equations are rigorous. At this point we make an assumption which introduces a slight inaccuracy, but greatly simplifies the treatment. We assume that the tube is operated under conditions such that the curve of  $i_b$  plotted against  $E_b$  is a straight line,

and that the slope of this line is not altered when it is displaced to right or left by a change of  $E_c$ . For the small changes of  $E_b$  and  $E_c$  involved in this work the assumption is a close approximation to the truth. The operation of the system on this assumption is shown graphically in figure 20. Two curves of  $i_b$  plotted against  $E_b$  for slightly different values of  $E_c$ , are shown side by side. The slope of these curves in the region used is such that  $\frac{di_b}{dE_b} = \frac{1}{r}$ ;  $r$ , may thus be

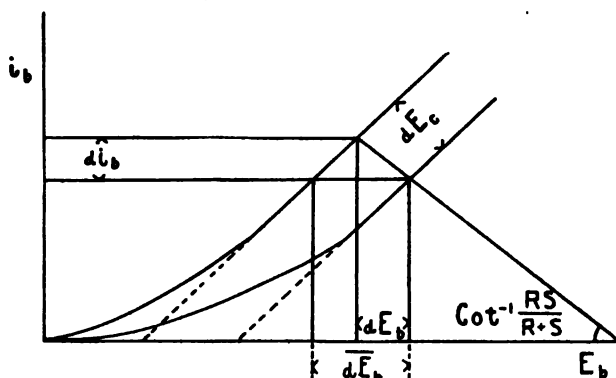


Fig. 20. Graphic representation of simplified mathematical analysis by Doctor Chaffee (see text).

regarded as the virtual resistance of the tube. The expression for the curve now becomes

$$i_b = \frac{1}{r} (E_b + \mu(E_c - E_o)). \quad (8)$$

In this expression  $r$ ,  $\mu$ , and  $E_o$  are constants.

Then  $\left(\frac{di_b}{dE_b}\right)_{E_c} = \frac{1}{r}$ , and  $\left(\frac{di_b}{dE_c}\right)_{E_b} = \frac{\mu}{r}$ .

We may now seek an expression for  $\frac{di_s}{dE_c}$ , the desired quantity;

$$\frac{di_s}{dE_c} = \frac{di_s}{di_b} \times \frac{di_b}{dE_c}.$$

\*The expression  $\left(\frac{di_b}{dE_b}\right)_{E_c}$  means  $\frac{di_b}{dE_b}$  when  $E_c$  is kept constant.

Substituting (7) in the above we have

$$\frac{di_a}{dE_c} = \frac{R}{R+S} \times \frac{di_b}{dE_c}.$$

The next step is to find  $\frac{di_b}{dE_c}$  under the conditions in the system.

$$di_b = \frac{\partial i_b}{\partial E_b} \cdot dE_b + \frac{\partial i_b}{\partial E_c} \cdot dE_c.$$

$$\frac{di_b}{dE_c} = \left( \frac{\partial i_b}{\partial E_b} \right)_{E_c} \cdot \frac{dE_b}{dE_c} + \left( \frac{\partial i_b}{\partial E_c} \right)_{E_b}.$$

Substituting the partial derivatives above this becomes:

$$\frac{di_b}{dE_c} = \frac{1}{r} \cdot \frac{dE_b}{dE_c} + \frac{\mu}{r}. \quad (9)$$

Now in this system any change in  $E_c$  causes a change in  $E_b$ . From the analysis of the system in accordance with Kirchhoff's laws as indicated in equations (1) to (5) any change in  $E_b$  must be correlated with  $i_b$  in accordance with the formula

$$\frac{di_b}{dE_b} = - \frac{R+S}{RS}. \quad (5)$$

Therefore any change in  $E_b$  produced by a change in  $E_c$  must cause a corresponding change in  $i_b$  which when plotted as in figure 20 will fall on a line whose slope is indicated by  $\frac{di_b}{dE_b}$ , i.e., the cotangent of whose angle with the horizontal is  $-\frac{R+S}{RS}$ . Let  $\overline{dE_b}$  be the differential of  $E_b$  when  $i_b$  remains constant, i.e., the change of  $E_b$  required to restore  $i_b$  to its original value when it has been altered by a change in  $E_c$ ;  $\overline{dE_b}$  will then represent the horizontal distance between the two curves. We shall use  $dE_b$  express the actual differential of  $E_b$  in the system as resulting from a change of  $E_c$ .

By definition

$$\frac{\overline{dE_b}}{dE_c} = \left( \frac{\partial E_b}{\partial E_c} \right)_{i_b} = - \frac{\frac{\partial i_b}{\partial E_c}}{\frac{\partial i_b}{\partial E_b}} = - \frac{\frac{\mu}{r}}{\frac{1}{r}}.$$



Therefore 
$$\frac{\overline{dE_b}}{dE_c} = -\mu. \quad (10)$$

By inspection of figure 20 it is evident that

$$\frac{\overline{dE_b} - dE_b}{di_b} = -r. \quad (11)$$

Combining this with (5) we get

$$\frac{\overline{dE_b} - dE_b}{di_b} \cdot \frac{di_b}{dE_b} = \frac{(R+S)r}{RS}$$

Simplifying this equation and adding 1 to each side,

$$\frac{\overline{dE_b}}{dE_b} = \frac{(R+S)r + RS}{RS}. \quad (12)$$

Combining this with (10) we get

$$\frac{dE_b}{dE_c} = -\frac{\mu RS}{(R+S)r + RS}. \quad (13)$$

Substituting this expression in (9) and reducing we get

$$\frac{di_b}{dE_c} = \frac{\mu(R+S)}{(R+S)r + RS}. \quad (14)$$

Finally by combining this with (7) we obtain the desired formula

$$\frac{di_s}{dE_c} = \frac{\mu R}{(R+S)r + RS}. \quad (15)$$

This equation enables us to calculate the quantity  $\frac{di_s}{dE_c}$ , upon which amplification depends, in terms of two known resistances and two constants which may be obtained from the characteristic curves of the tube, and these curves may readily be obtained by direct experiment. Evidently to make this expression large we should choose large values of  $\mu$  and  $R$ , and small values of  $r$  and  $S$ . But whereas the value of the expression is directly proportional to  $\mu$ , its increase with increase of  $R$  is relatively slight.

*Experimental.* Most of the experiments made in order to determine the method of obtaining the maximum amplification were performed before the mathematical analysis by Doctor Chaffee had been developed. The end result being a function of four independent variables, it was difficult to isolate the effect of varying each quantity by itself. A large number of experiments was necessary. In order to make the basis of comparison uniform we kept the resistance  $R_n$ , representing the nerve, constant at 10,000 ohms. It has already been mentioned that with the tubes first used, when an initial negative grid potential amounting to 1.5 volts or more was applied, the resulting excursion of the string became independent of the value of this resistance. Therefore the insertion of  $R_n$  under this condition became unnecessary. Early in the experiments we found that considerably greater amplification was obtained with a negative grid potential than without. Thereafter all experiments bearing on maximum amplification were made with at least 1.5 volts negative grid potential. For the same reason of uniformity of comparison it was necessary to use the same string throughout a series of experiments, or at least to keep the string resistance constant. Most of the earlier quantitative comparisons were made with string  $G$ , having a resistance of 20,000 ohms. Later this string lost its conductivity, and we were left to complete the experiments with only string  $B$ , having a resistance of 910 ohms. Therefore in order to make the experiments quantitatively valid we introduced a resistance of 19,000 ohms in series with string  $B$  to make the resistance of that part of the circuit the same as it had been with string  $G$ . In a few experiments we placed 20,000 ohms instead of 19,000 ohms in series with string  $B$  for convenience. In comparing results, amplification was defined as the ratio between the excursion of the string when connected with the electron tube, caused by placing a given change of potential (one or two millivolts), on the grid, and the excursion obtained by placing the same difference of potential in series with the string and the resistance  $R_n$  arbitrarily chosen to represent the resistance of a nerve. Clearly the amplification with a given arrangement depends on the magnitude of resistance selected to represent the nerve, since as already stated this magnitude has no effect on the excursion obtained with the electron tube under the best conditions of operation, whereas it makes a great difference in the excursion obtained without the tube. In giving figures for amplification the assumed value of  $R_n$  must be stated.

In order to simplify the problem as far as possible we decided to select a standard value of filament current and to use it in all quantitative experiments in order that this should not enter as a variable into the result. As already stated we found that the plate current increased rapidly with increase of filament current, until the latter reached a value of about 1.1 amperes, and after this, much less rapidly. Van der Bijl has shown (1, p. 175) that a tube should operate in the latter part of its curve; that is, under the condition that the plate current varies little with change of filament current. It is also easier to maintain uniform working conditions with the conductivity of the tube as insensitive as possible to changes of cathode temperature. On the other hand it is desirable not to operate the tube with too high a cathode temperature, since to do so shortens the life of the tube. For these reasons 1.20 amperes was selected as the standard filament current and used in a great majority of the experiments. In a few cases this was varied in order to determine the effect of doing so.

The values  $E$ ,  $R$  and  $E_c$  then remained to be studied with a view to choosing the optimum value of each. In our earlier experiments we varied  $E_c$ , keeping  $E$  and  $R$  constant, and then varied  $R$ , keeping  $E$  and  $E_c$  constant. The results were difficult to interpret, since by changing either one of these variables we changed indirectly the value of  $E_b$ , the actual voltage drop within the tube, thereby changing the tube resistance and producing a complex instead of a simple change of conditions. For instance, if  $E_c$  was changed, keeping  $E$  and  $R$  constant, the tube resistance was changed, and that portion of the total battery voltage,  $E$ , used up in the tube was correspondingly changed. The consequence was that we were not only studying a different part of the  $i_b$ ,  $E_c$  curve but a different part of the  $i_b$ ,  $E_b$  curve as well. In like fashion a change of  $R$  was complicated by a similar change of  $E_b$ . We finally decided to carry out some experiments keeping  $E_b$  constant at approximately 200 volts, the value of the plate potential recommended by Doctor Arnold as giving the best results with this type of tube. In this way we sought to determine under comparable conditions the best values of grid potential and total battery voltage, and as a corollary of the latter the value of the by-pass resistance,  $R$ . As nearly as we could judge from an effort to determine the influence of plate potential as distinguished from that of other factors in the earlier experiments, the best amplification was obtained in the neighborhood of 200 volts, although this value was by no means critical: amplification almost as great was obtained with values higher and lower than this.

Adherence to approximately 200 volts as a standard therefore appeared advisable. If the plate potential,  $E_b$ , is to be kept constant the problem of choosing the total battery voltage,  $E$ , becomes secondary to that of choosing the value of the by-pass resistance,  $R$ . That is, the choice of  $E$  is the means to the end of obtaining the desired ratio between by-pass resistance,  $R$ , and tube resistance,  $R_b$ .

Preliminary experiments had shown the greatest amplification to be obtained with values of  $E_c$  between  $-1.5$  and  $-4.0$ , and with values of the ratio  $\frac{R}{R_b}$  lying between  $0.4$  and  $0.9$ . In order to carry out a

complete experiment to show the effect of varying the ratio  $\frac{R}{R_b}$  at different values of grid potential, and of varying the grid potential at different values of the resistance ratio, we made a careful series of measurements of the tube resistance with the plate potential kept at 200 volts and the following values of grid potential:  $-2.0$ ,  $-2.5$ ,  $-3.0$ ,  $-3.4$  and  $-3.9$ . We selected the following values of the battery voltage,  $E$ : 278, 302, 322, 342, 362, as giving with the desired plate potential a series of resistance ratios ranging from  $0.4$  to  $0.9$ . Each series of observations was to begin with a battery voltage of 278. We therefore calculated from the above measurements of tube resistance the value of by-pass resistance,  $R$ , which for each value of grid potential would make the plate potential 200 volts with a battery voltage of 278. In this way each series of observations would start with the same value of  $E$ , the same value of  $E_b$ , and consequently the same resistance ratio  $\frac{R}{R_b}$ , namely  $0.4$ . With these calculations made

beforehand the experiment was begun, and on the first observation of each series, the plate current was read with the milliammeter. Thereafter each time the battery voltage was increased the resistance,  $R$ , was correspondingly increased just enough to maintain the same value of the plate current. In this way we could be certain that we were maintaining the same value of the plate potential,  $E_b$ , so long as the grid potential and filament current were not altered, and these were carefully controlled. Having completed such a series with a grid potential of  $-2.0$ , we began the second series with a grid potential of  $-2.5$ , using the same steps of battery voltage and beginning with the previously calculated value of  $R$ , noting the resulting value of the plate current and again keeping this constant throughout the series. In this way five series were carried out, but the last three were incom-

plete, since we had not resistances enough to maintain the higher ratios when the tube resistance became very high as a result of using a large negative grid potential. At frequent intervals during the experiment the apparatus was rearranged according to the standard wiring plan, and control observations were made with the current sent directly through the string. From these observations we plotted a curve showing the gradual change of string tension due to heating of the galvanometer, which enabled us to apply the necessary corrections to make the comparison valid. Owing to slight inaccuracies in the previous measurements of tube resistance and possibly to slight

TABLE 1

$E_c$	$R/R_b$				
	0.40	0.52	0.62	0.75	0.88
A					
-2.0	10.9	11.4	11.2	10.8	10.7
-2.5	10.8	11.0	11.1	10.9	10.5
-3.0	10.9	11.0	11.0	10.6	
-3.4	10.6	10.5	10.5		
-3.9	9.6	10.1			
B (corrected)					
-2.0	11.7	12.4	12.8	13.0	13.4
-2.5	11.6	12.0	12.7	13.1	13.1
-3.0	11.7	12.0	12.5	12.7	
-3.4	11.4	11.5	12.0		
-3.9	10.3	11.0			

changes in the behavior of the tube, we were unable to keep the resistance ratios precisely the same in each series of observations. But by means of the milliammeter readings of the plate current we were able to be sure that the plate potential remained constant during each series, and we were able to maintain it at nearly the same value through all of the series. The results are tabulated in table 1 A, the figure for amplification being given on the basis of 10,000 ohms as the value of  $R_n$ .

From this experiment it would appear that the greatest amplification occurred with a grid potential of about -2.0 volts and with a value of the by-pass resistance about half as great as that of the

tube. On the other hand it is evident from this and from the preliminary experiments that these values are not at all critical; in fact very nearly as great amplification is obtained with values of all the quantities varied over a wide range; in the case of grid potential all the way from  $-1.5$  to  $-4.2$ ; in the case of by-pass resistance all the way from equality with the tube resistance down to less than half of this value.

After the completion of this experiment the mathematical analysis given above was developed, and the results of experiments were thereupon compared with the calculated values of amplification based on the formula deduced by Doctor Chaffee,  $\frac{di_s}{dE_s} = \frac{\mu RS}{(R + S)r + RS}$ . In making these calculations the values of  $\mu$  and  $r$  were obtained by making careful measurements of the tube characteristics, plotting curves of these on coördinate paper and measuring the slopes of the curves at the proper points.

The comparison of observed and calculated values showed in general a striking agreement. In those experiments in which no initial negative grid potential was applied and in which therefore the grid circuit absorbed current, there was a wide discrepancy between the calculated and observed values. But inasmuch as the mathematical analysis takes no account of such action in the grid circuit this particular discrepancy is to be expected. It is furthermore unimportant, since the amplification is much less under this condition than with a negative grid potential. Therefore only those results in which there was a negative grid potential, and the system operated as an electrometer, need be considered. In all such cases the greatest discrepancy found amounted to only about 15 per cent; in a majority of observations it was less than 5 per cent; in some observations there was practically none. On the other hand in the experiments just described in which conditions were most carefully controlled with reference to constancy of plate potential, we found an inverse correlation which, though slight, was constant, and therefore led us at first to think that the formula failed to deal adequately with all the factors. According to the formula the 66 per cent increase in by-pass resistance between the second and fifth observations in the top row of table 1 A, in which grid potential was kept at  $-2.0$  volts, should have caused an increase in amplification amounting to 8 per cent. In reality it caused a decrease in amplification amounting to 7 per cent. Similarly in the second series when the grid potential was kept constant at  $-2.5$  volts a 60 per cent increase in

by-pass resistance, instead of causing the calculated 6 per cent increase in amplification, did cause a 4 per cent decrease in amplification. As stated above, in each of these comparisons grid potential remained constant, and plate potential was kept constant with the aid of the milliammeter in the plate circuit. Therefore, since we were working at precisely the same point in the characteristic curve of the tube, the greatest source of inaccuracy, viz., the determination of  $r$  and  $\mu$  depending on the slopes of the characteristic curves, was completely eliminated.

An explanation of the apparent discrepancy was found in the leakage of the condenser. It has already been noted that leakage occurred and that the resistance of fifteen one-microfarad condensers connected in parallel, (the arrangement regularly used) amounted to 32 megohms. When the voltage drop in  $R$  is increased, as in this experiment, by increasing the resistance ratio and with it the total battery voltage, the difference of potential between the condenser plates is increased and with it the current in the string due to leakage. This current is great enough to place the string under appreciably increased tension on account of displacement. This increased tension reduces the excursion resulting from a given current, and the greater the displacement the greater will be the reduction. In this way it seemed probable that the increase in amplification which should have resulted from increasing the ratio  $\frac{R}{R_b}$ , was more than nullified by the concomitant increase

in string tension. An experiment was made to test this point. The string was adjusted to the same initial tension that was used in the experiment described, and then deflected by a current of approximately the same magnitude as that resulting from the condenser leakage in the case of the lesser voltage drop. A small difference of potential was then applied and the excursion measured. Then the string was further deflected by a current as great as that leaking through the condenser under the greater voltage, and the excursion resulting from the same difference of potential was measured. The decrease of sensitiveness caused by the smaller displacement amounted to 17 per cent; the decrease caused by the larger displacement, 26 per cent. A correction for this difference applied to the amplification as observed in the experiment under consideration, sufficed to change the apparent loss of amplification to a gain of almost exactly the right amount to correspond with the calculated value. The agreement between observed and calculated values was thus brought well within the limits of observational

error. The experimental verification of Chaffee's formula thus became as complete and satisfactory as the experimental conditions rendered possible.

In table 1 B is shown the result of the experiment as modified by the corrections for change of string tension due to displacement. This shows the amplification which presumably would have occurred in this experiment had there been no leakage in the condenser.

In table 2 are shown a number of calculated and observed values of  $\frac{di_s}{dE_s}$  from experiments with different values of  $\mu$ ,  $r$ ,  $R$  and  $S$ . The

TABLE 2

$\mu$	$r$	$R$	$S$	$\frac{di_s}{dE_s} \times 10^4$	
				Calculated	Observed (corrected)
35.7	71,500	50,000	910	4.85	4.95
40.0	67,000	50,000	20,000	3.51	4.05
37.0	62,000	50,000	20,000	3.47	3.30
41.4	69,000	50,000	20,000	3.55	3.83
40.0	71,000	100,000	20,000	3.80	3.80
40.0	71,000	50,000	20,000	3.35	3.42
39.0	65,000	50,000	20,000	3.51	3.75
39.0	65,000	100,000	20,000	3.98	4.02
39.0	65,000	120,000	20,000	4.07	4.11
40.5	69,000	60,000	21,000	3.55	3.99
40.5	69,000	100,000	21,000	3.88	4.30
41.7	71,000	72,000	21,000	3.70	3.85
41.7	71,000	114,000	21,000	3.97	4.23

observed values are corrected for string displacement. They illustrate the agreement between prediction and result; and considering the difficulty in obtaining accurate values of  $\mu$  and  $r$ , they are quite satisfactory.

#### PRACTICAL DEDUCTIONS

Since the formula for amplification  $\frac{di_s}{dE_s} = \frac{\mu R}{(R + S)r + RS}$  is experimentally proved valid, it should be a simple matter to lay down practical rules for obtaining maximum amplification. Four distinct quantities are involved and may be dealt with in turn.

If  $r$  could be considered by itself we should want to make it as small as possible since it appears only in the denominator; that is, we



should want to choose the steepest part of the curve correlating  $i_b$  with  $E_b$  (in fig. 10 A). But since  $\mu$  is determined in part by  $r$  we must consider the two together. Clearly  $\mu$  should be made as large

as possible. But  $\mu$  is defined as equal to  $\frac{\left(\frac{di_b}{dE_c}\right)_{E_b}}{\left(\frac{di_b}{dE_b}\right)_{E_c}}$ ; that is, it is the

ratio between the slope of the  $i_b$ ,  $E_b$  curve and the slope of the  $i_b$ ,  $E_c$  curve, or the relative sensitiveness of plate current to changes in grid potential as compared with its sensitiveness to plate potential. In other words it depends not only on the steepness of the  $i_b$ ,  $E_c$  curve but also on the lack of steepness of the  $i_b$ ,  $E_b$  curve. Therefore making  $r$  small, while it serves to reduce the denominator of our expression, also reduces the value of  $\mu$  and therefore at the same time reduces the numerator. As already stated, the experimental evidence shows that the value of plate potential,  $E_b$ , is not critical as regards amplification. Very nearly the same results are obtained with values all the way from 150 to 250 volts. Considering both convenience and efficiency, a value between 160 and 200 volts is to be recommended.

With the value of  $r$  thus approximately established,  $\mu$  should be made as large as possible. This means we should seek the steepest part of the  $i_b$ ,  $E_b$  curve. But this leads us into the region where  $E_c$  is positive, and it has already been shown that unless  $E_c$  is made negative the grid circuit absorbs current, and in that way detracts from the amplification. We should then seek the steepest part of the curve where this will not occur. The results shown in the table appear to substantiate this view, in that the greatest amplification here is obtained with the smallest negative value of grid potential,  $-2.0$  volts. On the other hand, in other experiments with the same tube under otherwise closely similar conditions we have obtained just as great amplification with a grid potential of  $-3.0$  volts; and in some cases almost as great amplification with  $-4.0$  volts grid potential. It is therefore evident that with this tube the variations in  $\mu$  due to varying grid potential from  $-1.5$  to  $-4.0$  are not large, and do not make much difference in the resulting amplification.

These figures must not be relied on for all tubes of this type. A new D-tube, already referred to in footnote 4, was found to absorb current with a grid potential of  $-1.5$  volts; it was necessary to use as much as 3 volts negative bias to render the excursions independent of

input resistance,  $R_a$ , and to bring the tube to its point of maximum amplification. Each tube should be tested in this respect before it is used. In practice the most convenient arrangement is the insertion of either one or two dry cells (according to the requirements of the individual tube) in the input circuit with the negative terminal connected to the grid.

With regard to by-pass resistance,  $R$ , it is seen that when allowance is made for the effect of string displacement due to condenser leakage, an increase in amplification is obtained by increasing its value, provided the total battery voltage is increased at the same time enough to keep the actual plate potential the same. However, the increase in amplification after the by-pass resistance has attained a value of 60 per cent of the tube resistance is very small, and a practical limit is placed on its further increase by the undesirability of installing a battery of much more than 300 volts for so slight a gain as is to be had in this way. The correct value may be readily determined after the battery voltage,  $E$ , is established and the tube resistance,  $R_b$ , has been measured at a plate voltage of about 200 with the negative grid potential found necessary to prevent the grid circuit from taking current. The formula is  $R = \frac{R_b(E - E_b)}{E_b}$ . Assuming that the tube is to be operated at approximately 200 volts,  $E_b$ , the necessary data are provided.

The value of  $S$  is of some importance in determining the amplification; larger excursions are obtained for a given impressed e. m. f. with a low resistance string than with a high resistance string. On the other hand, on account of large resistances necessarily involved in this system this advantage is less than it is in the case of direct recording, except when the tissue is one of very high resistance. Furthermore, in the choice of a string the main consideration—lightness and correspondingly small inertia, which enable a string to respond rapidly to currents of brief duration—is just as important when the string is used with the electron tube as without. The string will therefore be selected with reference to its availability for nerve work in general, and may be regarded as a constant in this system, and not as a variable to be selected.

In some experiments the effect of varying the cathode temperature was tested. Although most of the records were made with a filament current of 1.20 amperes, some were made with 1.25 and some with 1.10. Those made with 1.25 amperes showed little difference in amplification

from those with 1.20. Reducing the filament current to 1.10 amperes in the tube first used caused a slight but appreciable reduction in amplification. For example, in one experiment with the following values: filament current, 1.10;  $E_c$ , -1.5;  $E$ , 270;  $R$ , 50,000 ohms;  $E_b$ , 185 volts; and a resistance ratio of 0.46, the amplification on the basis of 10,000 ohms as the value of  $R_n$ , was 10.3;—the greatest amplification on this basis obtained with any combination (not correcting for string displacement) was 11.5. But with the last tube used reduction of filament current below 1.15 amperes caused a marked loss in amplification. In general it is advisable for the sake of the resulting increase in the length of life of the bulb, to use it at the lowest filament temperature that will give satisfactory amplification.

In choosing the battery voltage,  $E$ , to be used when the installation was made permanent, we decided on 135 cells as being exceedingly convenient and at the same time efficient. Forty-five lead storage cells is the maximum number which, connected in series, can be charged efficiently from 110-volt power mains; 45 cells can be conveniently arranged in trays by placing them in nine rows of five cells to a row. Three such trays can be charged in parallel, and when connected in series will give at least 275 volts. As indicated above this voltage is sufficient to operate the tube at very near its maximum efficiency. In practice we have found that our battery when freshly charged has 300 volts on open circuit.

In an earlier section it was pointed out that inasmuch as the electron tube under proper conditions operates as an electrometer, measuring the difference of potential arising in the tissue irrespective of the resistance, the amplification of galvanometric excursion obtained by means of it, depends on the resistance of the tissue, since the excursion obtained with the galvanometer alone depends on tissue resistance as well as on electromotive force. In other words, since a large tissue resistance will reduce the response of the unaided galvanometer to a given e. m. f. but will not reduce the response of the galvanometer when working with an electron tube, a comparison between the two arrangements will show a greater gain in the case of a large resistance than in that of a small one.

This fact is shown quantitatively in figure 21. Here the amplification is plotted for each of the three strings against the value of  $R_n$ , expressed in thousands of ohms. The curve for string  $G$  (resistance 20,000 ohms) is plotted on the basis of an amplification of 11.4 (corrected) at 10,000 ohms tissue resistance, this being a value obtained

in a number of experiments and very nearly the greatest value obtained at all,—in one experiment Chaffee's formula agreed exactly with observation in giving this value. The curves for strings *B* and *H*, with resistances of 910 and 12,000 ohms, are based on the calculated amplification under those experimental conditions in which this precise agreement was found; that is, the proper quantity was substituted for 20,000 as the value of *S* in the formula.

It is notable that as mentioned earlier, amplification is replaced by reduction with the low resistance string when the tissue resistance

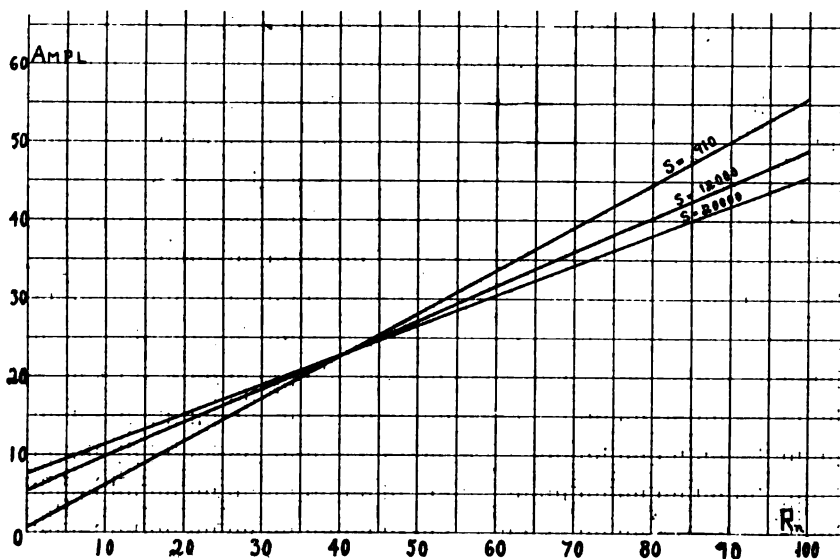


Fig. 21. Amplification (ordinates) plotted against tissue or input resistance,  $R_n$ , (abscissae) in thousands of ohms, for the three strings used in experiments, and for the particular case in which  $\mu = 40$ ,  $r = 71,000$  and  $R = 100,000$ .

becomes very small. With string *B* amplification ceases when  $R_n$  is reduced to about 1000 ohms. On the other hand with a tissue resistance of 42,000 ohms the amplification is identical with all three strings, and with greater tissue resistance the string of lowest resistance amplifies the most. With a tissue resistance of 100,000 ohms we obtain with string *B* a 55-fold amplification, whereas with the 20,000-ohm-string we obtain 45-fold amplification. These curves are only valid for the particular values of  $\mu$ ,  $r$  and  $R$  selected in this case. We have found that with a different choice of these values different curves are

obtained, similar in character and again all crossing at a point, but at a different point denoting a different value of  $R_n$ . Below this point the amplification is always greatest with the string of highest resistance; above it the inverse relation always holds. The curves are of necessity straight lines. It should be understood in estimating from these curves the relative merits of low and high resistance strings that the gain of amplification is not an absolute quantity, but only relative; it is merely the gain in excursion over what would be obtained in recording under similar conditions without the electron tube.

#### COMPENSATION FOR CONDENSER LEAKAGE

In an earlier section it was stated that in most of our experiments we met the problem of string displacement due to condenser leakage, by simply moving the fiber case laterally and thus bringing the string

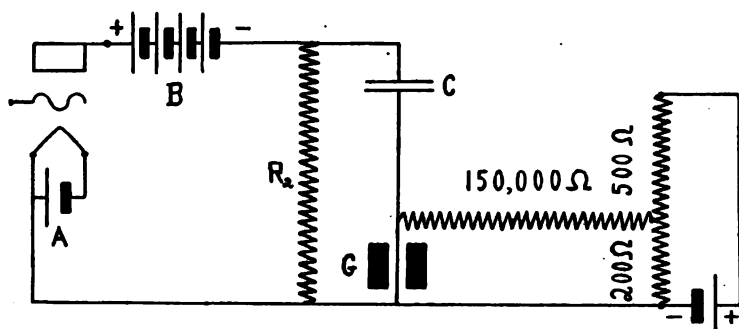


Fig. 22. Method of compensating for condenser leakage, proposed by Doctor Williams.

back into the center of the field. At the suggestion of Dr. H. B. Williams, an electrical method of compensation was tried and found satisfactory. A dry cell was connected with a resistance of 700 ohms. The negative pole of the dry cell was connected with the grounded end of the string. From a point in the resistance separated from this terminal by 200 ohms, connection was made with the other end of the string through the resistance of a graphite line drawn on ground glass, having a resistance of about 150,000 ohms. The arrangement is shown in figure 22. In this way enough current is tapped off from the dry cell circuit to neutralize the current in the string due to condenser leakage, and yet the resistance of the shunt circuit around the string is so much

higher than that of the string itself that it does not act to any considerable extent as a short-circuit. The combination described approximately balanced the condenser leakage, so that when the protective shunt was opened very little displacement occurred. The amplification with this arrangement was measured and compared immediately afterward with the amplification obtained with exactly the same values of the usual constants and with the ordinary method of bringing the string into the field by moving the fibre case. The excursion obtained with electrical compensation was slightly the larger of the two. This shows that if there is any loss of efficiency in this method it is less than the apparent loss of efficiency caused by the increased tension due to the usual displacement. If a standard value of each of the variables is adopted as a matter of permanent installation it is a simple matter also to keep permanently installed a dry cell with the necessary resistances, and to effect electrical compensation by the simple throwing of a single switch. By tapping from the compensating circuit through a still larger resistance, say a megohm, there would be practically no loss of efficiency.

#### PROPORTIONALITY OF EXCURSION TO INPUT VOLTAGE

For certain purposes it is desirable to know whether our system in amplifying gives a faithful record of the relative values of the disturbances amplified. That is, we should ascertain whether an e. m. f. of 2 millivolts applied to the grid will produce just twice as great an excursion of the string as 1 millivolt. In some experiments this point was put to test. In some cases the excursions resulting from 1 millivolt and 1.5 millivolts were compared in quick succession; in others comparisons were made between 1 and 2 millivolts. The proportionality regularly was good; in most observations it was accurate to within 2 per cent, and in no case was the value out of proportion by more than 4 per cent. This is within the limits of experimental and observational error.

#### APPLICATION TO PHYSIOLOGY

In order to put the electron tube method to the practical test of recording action currents, experiments were made with the sciatic nerves of both cats and frogs, and with the muscles of the human forearm led off through the skin by the usual method employed in making electromyograms (9).

The experiments on nerves will be described first. The resistance of a given length of a frog's sciatic nerve is very much greater than the resistance of the same length of a cat's sciatic nerve. Therefore the action current in the frog's nerve should be susceptible of far greater amplification than that in the cat's nerve, since the resistance in the tissue serves to reduce the current obtainable in the galvanometer when directly connected with the nerve, whereas we have seen that this resistance causes no reduction in the excursion of the galvanometer when used with the electron tube.

A preliminary experiment was made with a cat's nerve, using the heavy string (string *B*). This was done before the best values of the variables for amplification had been found. The result showed amplification, but not as great as was obtained under the more favorable conditions developed later. After the experiments on maximum amplification had been completed and the permanent installation had been arranged as described above, an experiment was made to illustrate the amplification of the monophasic action current in a frog's nerve; in this experiment the new string, *H*, with a resistance of 12,000 ohms, was used. The sciatic nerve of a good-sized frog was placed in a moist chamber with stimulating electrodes on the central end, and the distal end laid across non-polarizable boot electrodes 15 or 20 mm. apart. The nerve was crushed at a point midway between the boot electrodes to render the response monophasic. The resistance of the section of nerve and the electrodes in circuit with the string was measured by substitution, and found to be 85,000 ohms at the beginning of the experiment, rising as a result of drying to over 100,000 ohms at the end. Such a high resistance as this should prove favorable to great amplification, as indicated above. Specifically, with the combination of tube constants employed, which had previously shown 10.4-fold amplification with 10,000 ohms for  $R_n$  and a string resistance of 20,000 ohms, there should be with a nerve of 85,000 ohms resistance and a string of 12,000 ohms, a 40-fold amplification. In practice it was found that a maximal stimulus sent the shadow of the string off the film in every observation with the electron tube. The lack of constancy in the condition of the nerve made impossible any accurate quantitative comparisons in the case of submaximal stimuli, but as nearly as such comparison could be made it indicated about 45-fold amplification.

Records made in this experiment with and without the electron tube are shown in figure 23. In the first pair, (submaximal responses

produced by make shocks) the stimuli were of the same strength. In the second pair, since the maximal stimuli with the electron tube caused the excursion to exceed the width of the film, the response to a slightly submaximal break shock with the tube is compared with a maximal response with the unaided galvanometer. The speed of film is the same in all, but since the tuning fork causes interference with the electron tube arrangement, time is only shown on the records taken without it. It is noteworthy that the duration of the electrical disturbance in the nerve is much greater than one would suppose from the records made with the unaided galvanometer, amounting to as

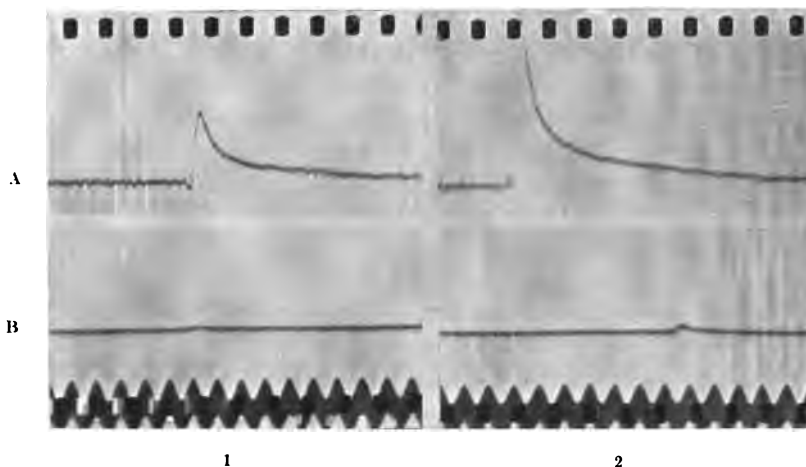


Fig. 23. Action currents in frog sciatic nerve (see text). String *H*. Tension, 1 cm. =  $4.16 \times 10^{-7}$  amp. A, electron tube arrangement; B, standard arrangement. Speed of film the same in all.

A1:— $I_A$ , 1.10;  $E$ , 300;  $E_b$ , 189;  $R$ , 60,000;  $E_c$ , — 1.5. Stimulus submaximal make shock.

A2:— $I_A$ , 1.10;  $E$ , 300;  $E_b$ , 207;  $R$ , 50,000;  $E_c$ , — 1.5. Stimulus, break shock, 8.0 Z units (Martin scale).

B1, Stimulus, make shock of same strength as A1. B2, Break shock, 34.5 Z units.

much as a tenth of a second. The observations were made at ordinary room temperature, i.e., about 20°C. In this experiment and in another under similar conditions, every action current recorded, whether produced by a weak or strong stimulus, showed this surprisingly long duration. These experiments were performed in December and January with one of the original D-tubes mounted rigidly in the



open air. Three experiments performed on frog nerves in late February and early March failed to show any such prolonged duration (see fig. 26 A). The experimental conditions were identical except that the new D-tube already mentioned, having slightly different characteristics, had been substituted for that used in the earlier experiments (this being broken), and the tube was now mounted in the sound-proof box. Tests with an artificial source of current fail to reveal anything in the new tube or its mounting which could account for this great change in the duration of the recorded action current. We are therefore led to the view that the difference in duration is physiological, possibly due to some seasonal change in the condition of the nerve.

It should also be noted in figure 23 that the direct action of the induction shock ("escape of current") appears in each record, preceding the action current (8, pp. 186-198). This was the case in all experiments with frog nerves even when the stimuli were subminimal. From its absence in the case of a nerve of low resistance (see fig. 26 B) we may infer that the prominence of this effect depends on a high resistance in the grid circuit.

In figure 24 are shown several electromyograms with and without the electron tube, some made with string *B*, a string of small resistance and large inertia, and some with string *H* having large resistance and small inertia. In the later of the two experiments when the high resistance string was used, care was taken to see that the contractions in each corresponding pair of observations were of the same strength, as indicated by the readings on the dynamometer which the subject gripped.

#### EXTRANEIOUS OSCILLATIONS

In figures 23 and 24 it will be seen that those records which were made with the electron tube and string *H* manifest fine oscillations at a frequency of about 305 a second. These are superimposed upon the coarser oscillations due to physiological activity. Similar oscillations were found in some of the earlier experiments with string *G* (also one of small inertia) as well as with string *H*, when used with the electron tube and an artificial source of current, but they were largest in the experiments with the frog's nerve. They were absent in all experiments made with the heavy string, *B*. On the other hand it is evident that they are not mere mechanical vibrations of the lighter strings, for they are only evident when the galvanometer is used in connection with the electron tube.

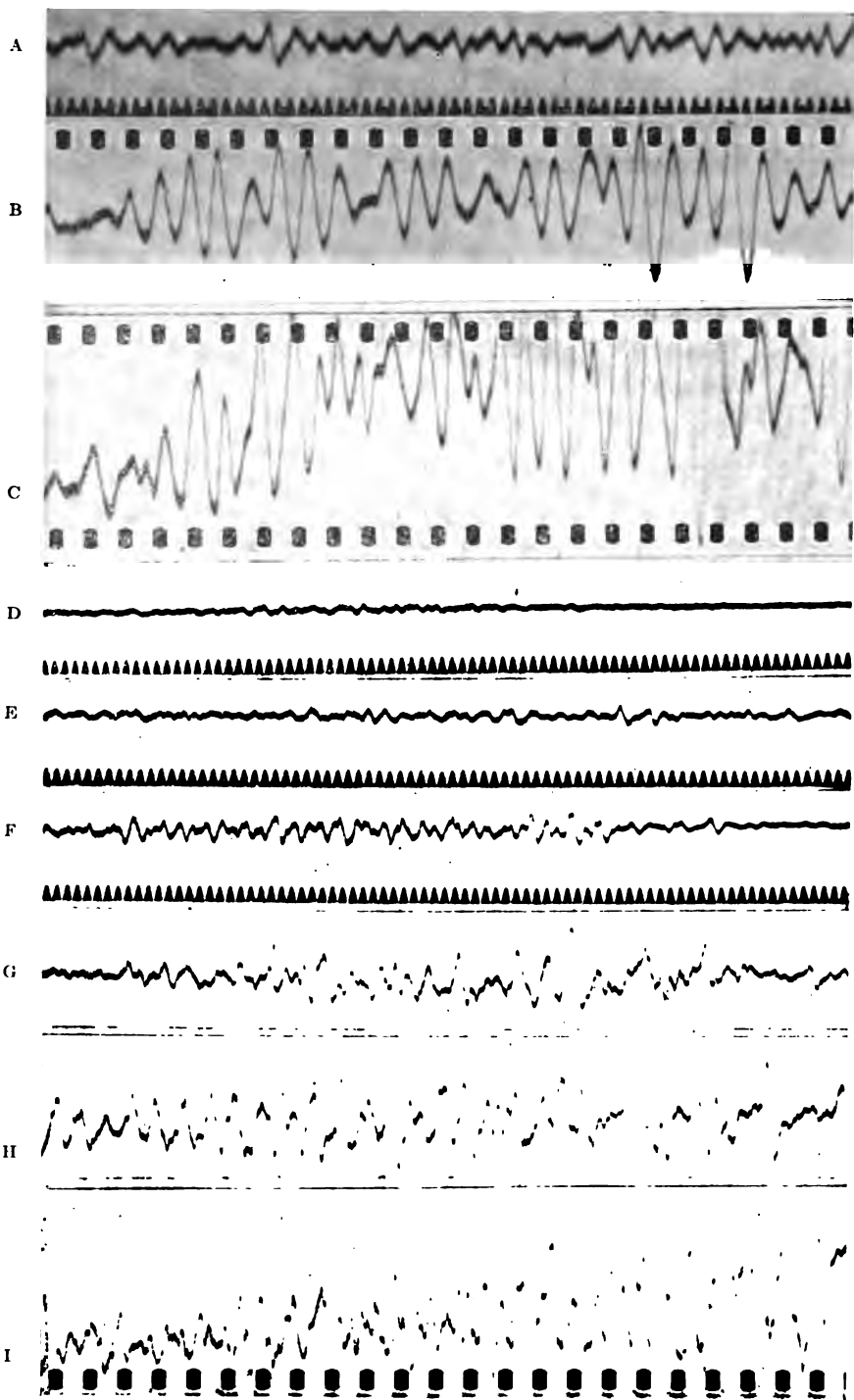


FIG. 24

A careful search was made for the cause of this disturbance. Since the electron tube arrangement had proved so sensitive to induction from the 110-volt motor used to drive the film in the older camera we were led to suspect that induction from other motors in the building might be the cause. Records were taken with all other motors in the building running and with all shut down, and the oscillations persisted under both conditions. The 12-volt motor which moved the film in the new camera was suspected. Records were therefore taken in which this motor was shut off while the clutch was in and the shutter open, so that the film continued to move as the motor slowed down. Still the oscillations persisted. The only significant fact noted in these experiments was that the oscillations were larger when a large resistance was included in the grid circuit, as was of course the case when the action current of the frog's nerve was being recorded. Finally, it was suggested to us by Dr. H. B. Williams that the cause of these oscillations might be modifications of the electron stream due to vibrations of one or more of the elements in the tube; filament, grid and plate. Such vibration is apt to result in these tubes from mechanical jarring or sound waves. This point was tested by tapping the tube gently while the film was being exposed; also by the sound of the human voice singing on the pitch indicated by the oscillations in the record; that is, about 305 a second. For comparison the sound of the voice singing on a pitch of 260 vibrations a second was tried. The effects of these three procedures are shown in figure 25. Evidently the tube has just such a natural period of vibrations and is extremely sensitive to mechanical jarring. It is also rather highly resonant to sound waves of that frequency.

In one experiment these vibrations were reduced to insignificant dimensions by greatly increasing the tension of the string and applying Einthoven's method of damping with a condenser across the string terminals (6). This remedy would answer fairly well for certain purposes, but it involves decreasing the excursion resulting from a given disturbance. Also it fails to give complete abolition of the oscillations.

Fig. 24. Electromyograms of human forearm flexors in voluntary contraction, subject squeezing dynamometer. A, B and C, string *B*; tension, 1 cm. =  $5.25 \times 10^{-7}$  amp. All others string *H*; tension, 1 cm. =  $4.16 \times 10^{-7}$  amp. A, strong contraction (strength not recorded), standard arrangement. B and C electron tube;  $I_A$ , 1.25;  $E$ , 238;  $R$ , 90,000;  $E_C$ , -3.0. B, weak contraction (10 kilos); C, strong contraction (28 kilos). D, E and F standard. G, H and I, electron tube;  $I_A$ , 1.10;  $E$ , 300;  $E_b$ , 207;  $R$ , 50,000;  $E_C$ , -1.5. D and G, dynamometer reading, 6 kilos; E, 10 kilos; H, 9.5 kilos; F and I, 20 kilos.

On the advice of Dr. H. B. Williams, a sound-proof box was constructed to contain the electron tube, and was suspended by the method of Julius (7). The box was made large enough to contain the tube with an air space of an inch or two between it and the lining. It was made of  $\frac{7}{8}$  inch wood, lined with  $\frac{1}{8}$  inch of lead and an inch of acoustic felt inside of that, except on the bottom where the socket was secured directly to the lead lining. Holes were drilled in the sides of the box to admit leads of flexible stranded bell-wire, which were soldered to the terminals of the tube socket. Outside of the box these leads were soldered to insulated and lead-sheathed wires by which the tube

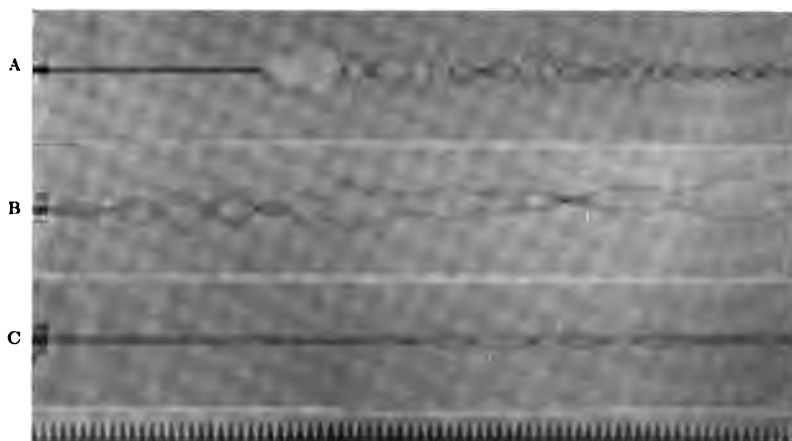


Fig. 25. Oscillations due to vibration of electron tube elements. String *H*. Tension 1 cm. =  $4.16 \times 10^{-7}$  amp. Condenser method.  $I_A$ , 1.10;  $E$ , 300;  $R$ , 50,000;  $E_c$ , -1.5. A shows effect of gently tapping tube. B, human voice singing on pitch of 305 a second, about a foot from tube. C, singing 260 vibrations a second, about the same intensity as in B. Time indicated as usual below.

was connected with the rest of the system, still in accordance with the wiring plan shown in figure 17, except that the milliammeter was eliminated as being unnecessary in the permanent installation. The lead lining of the box and the lead sheaths of the wires were grounded. The box was suspended by means of four steel springs such as are used for screen doors, attached to screw-eyes in the sides of the box at approximately the level of the center of gravity. The springs were hung from a bench secured solidly to the outside wall of the building, just under the galvanometer.

When the tube had been mounted in this box thus suspended, the same procedures that had caused the large oscillations shown in figure 25 were repeated. Loud sound waves from the human voice at a frequency of about 300 a second failed to produce any perceptible oscillations in the light string,  $H$ , which had vibrated so vigorously before. Screening from sound was evidently complete. The top of the bench directly over the suspension of the box was rapped vigorously, and this produced small vibrations whose amplitude was only a small fraction of that of the vibrations produced previously by rapping much more gently the table on which the tube had originally been mounted. When neither of these procedures was tried, the string appeared wholly free from the characteristic oscillations, whereas these had previously persisted, probably as a result of building vibrations, throughout the greatest quiet obtainable. The new mode of protecting against vibrations was thus found to be quite satisfactory.

More experiments were then carried out with both frog and cat nerves. In the case of frog nerves, having in circuit resistances of over 100,000 ohms, oscillations were still found, but they were much smaller than before the tube was protected, and they were irregular, having frequencies between 190 and 300 per second, with finer oscillations of about 600 per second superimposed. In the case of the sciatic nerve of a cat, the resistance of the part in circuit being about 10,000 ohms, the oscillations are almost if not wholly eliminated.

Figure 26 shows maximal monophasic action currents in both frog and cat nerves recorded with the electron tube in the sound-proof box. In each case there is shown for comparison the response of the same nerve to the same stimulus recorded with the unaided galvanometer. Calibration curves show the excursion of the string in each case with the same tension and with the same resistance in series when a constant voltage is applied. The irregular oscillations in the experiment with the frog's nerve may be seen in the figure.

Apparently the remaining oscillations are dependent on a large resistance in the grid (input) circuit. In seeking further light on their nature it was found that fairly regular oscillations of about 220 per second appeared whenever a large resistance,  $R_n$ , (e.g., 40,000 ohms) was introduced into the grid circuit as in figure 11. This is the same frequency with which the brushes shift contact on the commutator segments in the power house generator which supplies 110-volt direct current to the power mains throughout the building. Induction from this source is the probable cause of these oscillations. They are much

smaller than those which were traced to vibration in the tube. If the same resistance was introduced in the filament side of the input circuit, and if the greater part of  $R_1$  lay on the opposite side of the key from the point at which the input circuit is led off, there was a brief excursion of the string in the opposite direction from that usually seen, following the closure of the key and preceding the usual excursion such as is shown in figure 14. This initial excursion is due to a capacity effect which need not be analyzed here. It has no bearing on the recording of action currents. But it is mentioned because of the confusion which may result if the resistance used to determine whether the negative grid potential is sufficient to prevent the absorp-

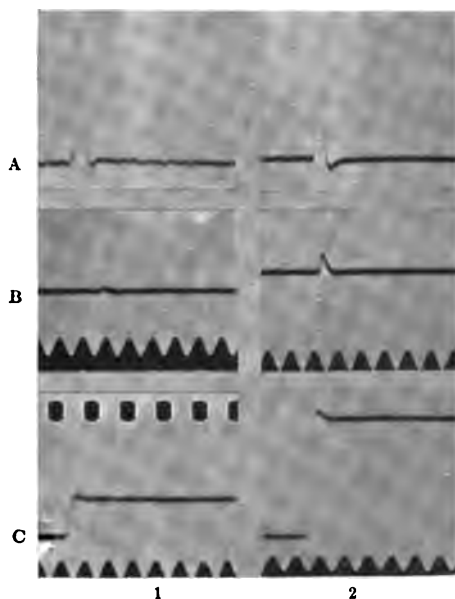


Fig. 26. Action currents recorded with tube in sound-proof box. String *H*. A, electron tube; B, standard; C, calibration curves.

1, frog sciatic nerve, resistance 115,000 ohms. Tension, 1 cm. =  $8.3 \times 10^{-7}$  amp. Stimulus in each case, 14.6 Z units.

2, cat sciatic nerve, resistance about 10,000 ohms. Tension, 1 cm. =  $8.3 \times 10^{-7}$  amp. Stimulus in each case, 46.2 Z units.

A1,  $I_A$ , 1.17;  $E$ , 300;  $R$ , 70,000;  $E_c$ , - 3.0.

A2,  $I_A$ , 1.06;  $E$ , 300;  $R$ , 70,000;  $E_c$ , - 3.0.

C1,  $\frac{60}{S + 100,000}$ . C2,  $\frac{30}{S + 10,000}$ .

Amplification in 2 is somewhat reduced by low value of  $I_A$ .

tion of current, is introduced in this side of the grid circuit, and if the primary resistance,  $R_1$ , is arranged in two parts with the key between them as was the case in most of our experiments.

The oscillations persisting in the records of frog nerves are probably in part due to the induction from the generator just mentioned. Their irregularity may indicate some further disturbance of physiological origin, or may be due to some extraneous cause not yet identified. At all events, the oscillations persisting in spite of the sound-proof box are insignificant as compared with a maximal action current in a nerve trunk; and with a resistance as low as is common in mammalian experiments they are imperceptible.

#### PRECAUTIONS

Several precautions in the use of the electron tube should be enumerated and emphasized. The most important of these concerns the avoidance of charging or discharging the condenser through the string. The rule is not to open or close any switch in the filament, grid or plate circuit, in short, anywhere in the electron-tube system, nor to make any adjustment in any part of the system while it is connected with the string galvanometer. All adjustments of current and voltage connected with the tube, and of by-pass resistance, should be made while the switch leading to the string is open and while the protective shunt (or equivalent short-circuit) is closed to enable the condenser to be charged from the B-battery. After all adjustments are made the switch leading to the string may be closed, and finally the protective shunt opened; but it should not be opened until at least 5 seconds after the closure of the switch which permits the charging of the condenser, for on account of the large resistance through which the charging current must pass, this time is required for the current to subside to a value which is safe to pass through the string. The string should be disconnected again before any switches in the rest of the system are opened. The least neglect of this rule will probably result in the loss of the string. There is one exception; the mere closing of the protective shunt, without disconnecting the string, is sufficient protection during minor adjustments of the filament current if made with sufficient caution.

If there is any possibility that one of the electrodes may be dislodged from its contact with the tissue under observation during an experiment, a shunt should be provided to prevent the grid circuit from

being broken. If a grid "bias" is used, the change in tube resistance which would result from breaking the grid circuit, would cause a current in the string so great as probably to destroy it. As a safeguard a graphite pencil line of about a megohm resistance, drawn on paper, has been connected across the leads to the non-polarizable electrodes, and has been found by experiment not to reduce measurably the excursion resulting from the action current of a nerve.

The filament current should be shut off when the tube is not in use. With the tube out of sight in a sound-proof box, one is apt to overlook this at the close of an experiment. The life of a tube is usually long, but not unlimited. In one case a tube was permanently impaired by accidentally leaving this current on until the battery was discharged.

Other precautions have been touched on in connection with the electrically induced oscillations caused by the Sandstrom kymograph motor and by the electrically driven tuning fork, and in connection with the oscillations traceable to vibration of the tube. Two distinct classes of disturbance are met with, those electrically induced and those of a mechanical origin. Two ways of dealing with the electrical disturbances are open,—prevention of the cause, and intervention between the cause and effect by shielding. It is desirable to avoid the use of electric motors in the vicinity of the system, and to minimize sparking from any source. To drive the film a low voltage motor completely encased in grounded metal is satisfactory. To record time a device which is free from the sparking of an electromagnetic tuning fork, should be used.

It has been found that rapid motion of the body close to the grid circuit, or shuffling of the feet produces marked excursions of the string. These are to be explained by the electrostatic induction in the grid circuit by the more or less charged body. On this account the experimenter should stand as still as possible during an observation. This disturbance is somewhat reduced by grounding the body of the experimenter. This has been effected by keeping the hand on the clutch which sets the film in motion, and which is grounded.

If, when a stimulating inductorium is set up near the electron tube system, the primary circuit of the inductorium is touched with the hand, an excursion of the string will result when the circuit is made or broken, even if there is no connection between the inductorium and the recording system (see p. 428). It is essential, therefore that if the stimulating key be operated by hand it should be done through an insulated handle.



It is important to see that all connections are good; they should be soldered as far as possible. A loose connection will introduce serious disturbance and may result in the loss of a string.

It is also very important that all batteries, especially the B-battery, should be in good condition. Dry cells which are nearly exhausted or storage cells in bad condition or bubbling as a result of too recent charging, may cause troublesome excursions of the string.

The danger of mistaking static effects or other extraneous disturbances for physiological effects is always present in the study of action currents with the string galvanometer. Amplification with the electron tube enormously increases this danger; one cannot be too careful to guard against it both by eliminating all possible sources of disturbance and by scrutinizing all results obtained with reference to latent period, dependence on vitality in the tissue studied, and any other possible criteria which may serve to verify the physiological origin of the current observed.

#### SUMMARY

1. The electron tube is a device wherein a stream of electrons emitted from a hot cathode in a vacuum and drawn to an anode (plate) by a high voltage battery, is modified by small changes of potential applied to a third electrode known as the grid, placed in the intervening space. In this way it serves as an amplifying relay and is commonly used as such in radio telegraphy.

2. This device offers great possibilities of amplifying such action currents in the nervous system as are too small to be recorded satisfactorily with the string galvanometer alone. When the string galvanometer is used to record the current amplified by this device it cannot be placed directly in the path of the high voltage current, as is done with the telephone, without destruction of the string. This must be protected from the direct current, and yet so connected with the system as to record changes in the electron stream.

3. Three methods of connecting the string with the tube in such a way as to afford the needed protection, were tried. These are designated the transformer method, the bridge method and the condenser method. They are illustrated diagrammatically in figures 3, 4 and 5. The condenser method was the only one which proved wholly satisfactory. It consists in placing a very large condenser in series with the string, and shunting the plate current by both through a resistance

of the same order of magnitude as the plate-to-filament resistance of the tube. By using a condenser with a capacity of as much as 15 microfarads the distortion due to gradual discharge of the condenser when the system is unbalanced becomes negligibly small in the case of action currents of brief duration. A permanent wiring arrangement was installed by means of which the electron tube system can be made available for the amplification of action currents in a tissue under observation, by merely throwing a few switches and making two or three adjustments, a process requiring less than two minutes. "D-tubes" loaned by the Western Electric Company, have been used in these experiments.

4. An effort was made to determine those values of filament-heating current, plate-battery voltage, grid potential, and by-pass resistance which would afford the greatest possible amplification. In general, amplification depends on a large proportional change in tube resistance for a given change in grid potential, together with other factors whose interaction is rather complex.

5. Complete mathematical analysis of the problem, using the empirically established formulae for tube characteristics, leads to insoluble expressions. Dr. E. L. Chaffee devised a method of obtaining an approximate formula for expressing in terms of two easily measurable quantities in the tube characteristics and of the resistance of the string and the by-pass resistance, amplification in any given case. The formula is  $\frac{di_g}{dE_g} = \frac{\mu R}{(R + S)r + RS}$ . In this  $i_g$  is the current in the string;  $E_g$  is grid potential;  $R$  is the by-pass resistance;  $S$  is the resistance of the string;  $r$  is the virtual resistance of the tube as determined by the reciprocal of the slope of the curve correlating plate current with plate potential;  $\mu$  expresses the sensitiveness of plate current to grid potential as compared with its sensitiveness to plate potential, in other words, the ratio between the slopes of the curves correlating plate current with grid potential and with plate potential respectively.

6. A large number of experiments in which the amplification was measured with a large variety of combinations of the quantities above enumerated, verified Doctor Chaffee's formula. Unless the grid is given an initial negative potential the grid circuit will absorb current and thereby detract considerably from the amplification. It was found that best results are obtained with a filament current of 1.1 to 1.2 amperes, with a negative grid potential of 1.5 to 3.0 volts (the larger value

being necessary with some tubes), with a plate voltage of approximately 200, and with a plate battery voltage as large as is convenient to install in the laboratory, the difference between plate and plate battery voltage being consumed in the by-pass resistance. This resistance should be at least half as large as the resistance of the tube, and preferably more than that. In practice the combination should depend on preliminary tests of the individual tube. Convenient and efficient arrangements with the D-tubes used have been within the following limits—filament current 1.1 to 1.2; grid potential  $-1.5$  or  $-3.0$  (one or two dry cells); plate battery voltage 300; by-pass resistance 50,000 to 80,000 ohms.

7. The use of paper condensers, which are most convenient when a very large capacity is desired, introduces sufficient leakage of current to cause a considerable displacement of the string. With the Cambridge string galvanometer the string may be simply brought back into the field by lateral adjustment of the fiber case. A somewhat better method is to tap off from a dry cell through a high resistance an equal and opposite difference of potential.

8. As nearly as we could measure it, the amplified current in the string galvanometer was strictly proportional to the e. m. f. applied to the grid circuit.

9. If the grid potential is kept negative with respect to the filament by a sufficient amount to prevent the flow of current in the grid circuit, the excursions of the string depend wholly on the e. m. f. impressed on the grid, and are independent of the resistance of the input circuit, i.e., with values of resistance at least up to 150,000 ohms. In this way the system operates as an electrometer rather than a galvanometer. The amplification, or ratio between excursions of the string with and without the tube depends both on the resistance of the input or tissue circuit and on the resistance of the string. The electron tube necessarily introduces high resistance into this system; therefore if the current to be recorded arises in a tissue of small resistance, and is recorded with a string of small resistance, the use of the electron tube will afford no amplification. But if either string or tissue has a large resistance there will be amplification. Given the resistance of the string and the amplification obtained with any given tissue resistance, the amplification obtainable with any other tissue resistance may be simply calculated. In the case of a frog's sciatic nerve having a resistance of 100,000 ohms between the leading-off electrodes, the amplification may be more than 50-fold.

10. Records have been made with the electron tube of the action currents in frog's nerve, and in human muscles during voluntary contraction, both with a high resistance string and with a low resistance string. These have been compared with action currents recorded directly with the unaided galvanometer under otherwise similar conditions. In the case of the frog sciatic nerve an amplification of more than 40-fold has been found.

11. When a motor operated on the 110-volt power mains was used to drive the film for photographic recording, oscillations were induced in the galvanometer through the electron tube system, which we were unable to prevent by screening. It proved necessary to record with a camera designed to work with a 12-volt motor completely encased in metal and run from a storage battery within the room. This camera has certain advantages over those previously used, and a brief description is included. Oscillations were also induced by an electrically driven tuning fork. These oscillations were probably traceable to high frequency currents arising in the electric spark and rectified by the tube. It was necessary to dispense with this method of recording time.

12. Other oscillations occurred in the galvanometer when connected with the electron tube system, which were traceable to vibrations in the elements in the tube, causing fluctuations in the electron stream. This difficulty was eliminated by installing the electron tube in a sound-proof box, suspended by the method of Julius. Smaller oscillations persisted in experiments with nerves of very high resistance, traceable probably to induction from the power mains. They are so small as to be insignificant, and were absent in experiments with tissues of medium resistance.

13. In addition to the precautions indicated in the last two sections, it is most important that the string be protected from the high voltages used in this system by careful avoidance of adjustments in any part of the system while the string is connected with it, and by allowing the condenser to become charged through a protective shunt or short-circuit before any connection with the string is made. Other confusing effects due to static electricity or bad connections or batteries in poor condition, must be carefully guarded against, if satisfactory results are to be obtained.

We wish to express our thanks to Dr. H. B. Williams, Dr. H. B. Arnold, Mr. E. H. Colpitts, Mr. S. W. Dean, Mr. Sewall Cabot and

Prof. G. W. Pierce for suggestions and advice, and especially to Dr. E. L. Chaffee for the mathematical analysis, and to Miss M. D. Ring for help in preparing the work for publication.

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## VARIATIONS IN THE RESPIRATORY DEAD AIR SPACE DUE TO CHANGES IN THE DEPTH OF BREATHING

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The variation which occurs in the capacity of the dead air space of the lungs because of changes in the tone of the bronchial muscles or in the amount of air inspired at each breath, is unsettled. In a previous paper of this series (1) it was shown that the dead air space has a fairly constant volume at ordinary depths of inspiration, and the great variation in the capacity of the dead air space found by Haldane (2) and by Henderson (3) and co-workers was not sustained, while the findings of Krogh and Lindhard (4) were in the main substantiated. These latter observers found evidence of a small increase in the capacity of the dead air space when maximal breaths were taken. While we were unable to obtain absolute evidence of even this small increase, we believe that our early results suggested such a possibility. Our experiments were free from many errors present in earlier work, but owing to the manner of combining our figures we obtained averages of the dead air space for all depths of inspiration. Later work by us indicated that, at moderate volumes of inspiration, the dead air space was a relatively fixed volume. Unfortunately, in the method employed we did not and could not use extreme volumes of inspiration. Moreover the spirometers we used had a dead air space of their own which, although we tried to eliminate it by filling with expired air before the observation, must have contributed toward making our results too high. In order to put this subject to further test, we have devised a method which we believe will, if properly carried out, give a fairly accurate measure of the capacity of the air passages at all depths of inspiration.

In this method, as in the former method, we used the mathematical formula given in our first paper (5). By it the carbon dioxide contents of a large and a small expiration following a measured inspiration are compared with the volume of air in a large and a small expiration in

a manner so that the air from the dead space may be determined. In the application of this formula to estimating the capacity of the dead air space at different lung volumes, we assume that the amount of carbon dioxide which is picked up by the definite volume of air inspired and expired quickly, following a forced expiration to residual air, is fairly constant in amount for a given state of bodily activity. That this is the case we have satisfied ourselves. We also assume that the carbon dioxide in the alveolar air collected for analysis has a uniform concentration. We admit that such is not the case actually, but for practical purposes of the experiment, providing the expirations compared do not differ by more than 400 cc., the assumption may be allowed.

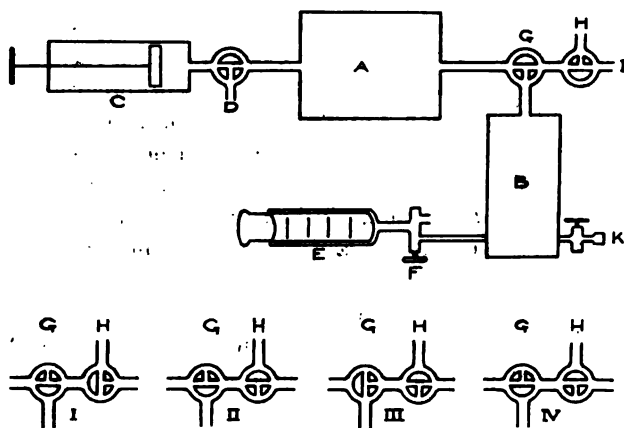


FIG. 1

It is also assumed that, given the same volume of inspiration, the capacity of the air passages will be the same in the different observations necessary to collect the required data. By progressively increasing the amount of air inspired, any variation of the dead air space due to stretching of the bronchial tree can be detected if such variations be greater than the experimental error of the method. The essential feature of this method is that a sharp, quick expiration to residual air be followed by a quick inspiration of a measured volume of air, and this followed by a quick expiration, the first portion of which is collected for measurement and analysis. The amount of air collected must be varied between 400 and 800 to 1000 cc. for best results.

Briefly the experimental procedure is as follows. Reference is to figure 1.

Bag *A* is a flat rubber bag capable of holding 5 liters of air. It connects with a three-way stopcock, *G*, which in turn connects with stopcock *H*, which has a mouthpiece, *I*. Bag *B* is a flat rubber bag of 1200 cc. capacity attached to cock *G*. *C* is an automobile grease gun, delivering 340 cc. of air at a stroke, and *D* a three-way cock for taking air into the gun and delivering it into bag *A*. *E* is a 100 cc. Luer syringe, and *F* a three-way cock attached to bag *B* for measuring the volume of air in bag *B*. *K* is a tube with cock for obtaining samples for analysis. The bags are flat and do not hold an appreciable amount of air when evacuated.

Bag *A* is filled with a measured amount of air by *C*. The cocks are then as shown in position 1. The subject is seated, and after five minutes of rest makes a quick forced maximal expiration. This must have followed a normal inspiration. Cock *H* is then turned to position 2, and a quick inspiration empties bag *A*. The breath is held while cock *G* is turned to position 3 and a forced expiration is made into bag *B*. In this case, however, cock *G* is quickly turned to position 4 during the expiration. By this means it is possible to limit the amount of air collected in bag *B*. The air in bag *B* is then analyzed accurately for its carbon dioxide content, and its volume measured by means of syringe *E*.

For each inspiratory volume it is necessary to make at least four observations, in which two small volumes and two somewhat larger volumes of the first portion of the expired air are collected in bag *B*.

The amounts of air collected in bag *B* and their carbon dioxide content are combined in the formula for the estimation of the dead air space as given in our previous papers. The formula reads: Volume of large multiplied by the volume of the small portions of expired air, multiplied by the difference in the percentage of carbon dioxide in the large and small portions of expired air, divided by the difference in the total volume of carbon dioxide found in the large and small portions of expired air, equals the volume of the dead air space.

The validity of using this method for obtaining the volume of the dead air space depends on certain factors: *a*, For each individual series of observations in which a given volume of air is inspired, the amount of carbon dioxide produced per unit of time must be constant during the experiment. For this reason it is absolutely necessary that the subject rest 3 to 5 minutes before each observation. *b*, The inspiration of the measured amount of air in the large bag must follow the deepest possible expiration, which in turn must have followed a normal inspira-



tion. This must obtain, since otherwise the amount of carbon dioxide in the lung air and the amount which is delivered by the blood to the lung air during the moment of observation would vary. *c*, The small bag, which collects the first part of the inspiration, must not contain any air except that which has been received from the first part of the forced expiration. *d*, The analysis of the carbon dioxide content of the air in the small bag must be very accurate, and the volume of the air collected in the small bag must be measured very accurately. *e*, The periods of time taken for the observations must be about equal, in order that the carbon dioxide eliminated by the blood be the same for each observation.

The results obtained on the subjects are given in tables 1, 2, 3 and 4, and are plotted as curves on cross-section paper, figure 2, the abscissae of which represent the volumes of air inspired and the ordinates the volumes of dead air space found with the respective inspiratory volume. It will be noted that when ordinary volumes of inspiration—1500 to 2500 cc. of air—are taken after a forced expiration (which amount is practically that which is ordinarily present in the lungs at the end of the normal inspiration), the variations of dead air space are very little but progressively increase; while with larger volumes of inspiration the dead air space increases from 100 to 115 per cent maximum. In other words, the increase in dead air space with the increasing volumes of inspiration is not a linear function to the depth of inspiration but a powered function. The explanation of this phenomenon no doubt rests in the fact that the small bronchioles contribute much to the volume of dead air space and are expanded somewhat during the inspiratory effort. The greater the inspiratory effort, the greater must be the negative pressure of the intra-thoracic pressure. Since the bronchi and bronchioles are made up of more or less elastic tissue, it is reasonable to suppose that their walls are dilated more or less proportionately to the degree of intra-thoracic pressure. Since the area of a circle varies as the square of its radius, it is apparent that a slight progressive increase in the diameter of the bronchioles will cause a relatively disproportionate and larger increase in the area of the cross section of the bronchioles.

The curves obtained very strongly suggest that this condition obtains to a greater or less degree during deep breathing. It is interesting to note that the maximum variation of dead air space, obtained when the deepest possible inspiration was made, is about 115 cc., corresponding closely to the figures obtained by Krogh and Lindhart (4). It

TABLE 1

*Subject R. G. Pearce, height 5 feet 9 inches, weight 178 pounds. Vital capacity about 4000 cc.*

EXPERIMENT NUMBER	AIR INSPIRED FROM BAG A	AIR EXPIRED INTO BAG B	CO <sub>2</sub> IN BAG B	VOLUME OF CO <sub>2</sub> IN BAG B
	cc.	cc.	per cent	
1	1700	660	3.50	23.00
2	1700	690	3.55	24.50
3	1700	390	3.10	12.10
4	1700	350	3.00	10.50
1	2380	685	2.90	19.80
2	2380	590	2.80	16.50
3	2380	303	2.25	6.80
1	3060	585	2.60	15.25
2	3060	383	2.15	8.20
1	3740	720	2.40	21.70
2	3740	655	2.85	18.60
3	3740	665	2.80	18.50
4	3740	395	2.10	7.80
5	3740	365	2.10	7.30

*When 1700 cc. of air are inspired:*

By combining 1 and 3 we have 94.5 cc. dead air space

By combining 1 and 4 we have 92.0 cc. dead air space

By combining 2 and 3 we have 97.5 cc. dead air space

By combining 2 and 4 we have 95.0 cc. dead air space

Average dead air space when 1700 cc. are inspired is 94.8 cc.

*When 2380 cc. of air are inspired:*

By combining 1 and 3 we find 104 cc. of dead air space

By combining 1 and 4 we find 101 cc. of dead air space

Average dead air space when 2380 cc. are inspired is 102.5 cc.

*When 3060 cc. of air are inspired:*

By combining 1 and 2 we have 143 cc. of dead air space

*When 3740 cc. of air are inspired:*

By combining 2 and 3 we find 201 cc. dead air space

By combining 3 and 5 we find 182 cc. dead air space

By combining 1 and 4 we find 184 cc. dead air space

By combining 1 and 5 we find 165 cc. dead air space

By combining 3 and 4 we find 196 cc. dead air space

By combining 3 and 5 we find 172 cc. dead air space

Average dead air space when 3740 cc. are inspired is 183 cc.

*Summary*

1700 cc. inspired gives dead space of 94.5 cc.

2380 cc. inspired gives dead space of 102.5 cc.

3060 cc. inspired gives dead space of 143.0 cc.

3740 cc. inspired gives dead space of 183.0 cc.

TABLE 2

*The following results were obtained on D. H. H. between 4:00 and 6:00 p.m., June 3, 1918, the subject being seated in a chair at rest. The experiment was the same as above, save that the expired air was collected in a small spirometer. The dead space of the apparatus when these data were collected was probably 20 cc. greater than with the improved method using the rubber bag B. His vital capacity was about 4000 cc.*

EXPERIMENT NUMBER	INSPIRED	EXPIRED SPIROMETER B	CO <sub>2</sub> SPIROMETER B	CO <sub>2</sub> SPIROMETER B
	cc.	cc.	per cent	cc.
1	1500	560	2.75	15.40
2	1500	525	2.70	14.20
3	1500	930	3.05	28.40
4	1500	1035	3.10	32.10
1	2100	735	2.70	19.80
2	2100	670	2.60	17.40
3	2100	1070	3.05	32.60
4	2100	1085	3.10	33.60
1	2900	1130	2.50	28.20
2	2900	1060	2.47	26.20
3	2900	500	1.80	9.00
4	2900	525	2.00	10.50
5	2900	525	1.85	9.70
1	3600	1035	2.20	23.20
2	3600	1125	2.32	26.10
3	3600	1105	2.33	26.80
4	3600	620	1.75	10.85
5	3600	630	1.75	11.00
1	4300	680	1.85	12.60
2	4300	675	1.75	11.80
3	4300	1125	2.25	25.30
4	4300	1085	2.15	23.30

*When 1500 cc. of air are inspired:*

By combining 1 and 4 we find 120 cc. dead air space

By combining 2 and 4 we find 121 cc. dead air space

By combining 1 and 5 we find 130 cc. dead air space

By combining 2 and 4 we find 121 cc. dead air space

Average dead air space when 1500 cc. are inspired is 123 cc.

*When 2100 cc. of air are inspired:*

By combining 1 and 3 we find 120 cc. dead air space

By combining 2 and 3 we find 129 cc. dead air space

By combining 1 and 4 we find 140 cc. dead air space

By combining 2 and 4 we find 139 cc. dead air space

Average dead air space when 2100 cc. are inspired is 132 cc.

*When 2900 cc. of air are inspired:*

By combining 1 and 3 we find 208 cc. dead air space

By combining 2 and 3 we find 206 cc. dead air space

By combining 1 and 4 we find 156 cc. dead air space

By combining 1 and 5 we find 208 cc. dead air space

By combining 2 and 5 we find 209 cc. dead air space

By combining 2 and 4 we find 166 cc. dead air space

Average dead air space when 2900 cc. are inspired is 192 cc.

*When 3600 cc. of air are inspired:*

By combining 1 and 4 we find 238 cc. dead air space

By combining 1 and 5 we find 245 cc. dead air space

By combining 2 and 4 we find 261 cc. dead air space

By combining 3 and 4 we find 245 cc. dead air space

Average dead air space when 3600 cc. of air are inspired is 247 cc.

*When 4300 cc. of air are inspired:*

By combining 1 and 3 we have 241 cc. dead air space

By combining 2 and 3 we have 280 cc. dead air space

By combining 1 and 4 we have 201 cc. dead air space

By combining 2 and 4 we have 253 cc. dead air space

Average dead air space when 4300 cc. of air are inspired is 243 cc. In this case 3800 cc. was about the vital capacity.

*Summary*

1500 cc. inspired gives dead space of 123 cc.

2100 cc. inspired gives dead space of 132 cc.

2900 cc. inspired gives dead space of 192 cc.

3600 cc. inspired gives dead space of 247 cc.

3800 cc. (maximum) gives dead space of 243 cc.

This is given in form of curve in which 20 cc. are deducted from each figure because of the dead space present in the spirometer. This figure is an approximate one only for the actual dead space of the Krogh spirometer is difficult to measure accurately.

TABLE 3  
*Subject R. G. Pearce*

EXPERIMENT NUMBER	AIR INSPIRED FROM BAG A	AIR EXPIRED INTO BAG B	CO <sub>2</sub> IN BAG B	VOLUME OF CO <sub>2</sub> IN EXPIRED AIR
	cc.	cc.	per cent	
1	1700	830	3.05	25.40
2	1700	925	3.00	27.75
3	1700	560	2.80	15.70
4	1700	455	2.70	12.25
1	2680	720	2.30	16.55
2	2680	520	2.10	10.90
3	2680	470	2.00	9.40
4	2680	340	1.75	5.95
1	3740	750	2.30	17.40
2	3740	663	2.25	14.90
3	3740	430	1.75	7.55
4	3740	425	1.75	7.45

*When 1700 cc. of air are inspired:*

By combining 1 and 3 we find 120 cc. dead air space

By combining 1 and 4 we find 101 cc. dead air space

By combining 2 and 3 we find 85 cc. dead air space

By combining 2 and 4 we find 82 cc. dead air space

Average dead air space when 1700 cc. are inspired is 97 cc.

*When 2680 cc. of air are inspired:*

By combining 1 and 3 we find 142 cc. dead air space

By combining 1 and 4 we find 128 cc. dead air space

By combining 2 and 4 we find 125 cc. dead air space

Average dead air space when 2680 cc. of air are inspired is 132 cc.

*When 3740 cc. of air are inspired:*

By combining 1 and 3 we find 176 cc. of dead air space

By combining 2 and 3 we find 176 cc. of dead air space

By combining 1 and 4 we find 191 cc. of dead air space

By combining 2 and 4 we find 189 cc. of dead air space

Average dead air space when 3740 cc. of air are inspired is 183 cc.

*Summary*

1700 cc. inspired gives a dead air space of 97 cc.

2680 cc. inspired gives a dead air space of 132 cc.

3740 cc. inspired gives a dead air space of 183 cc.

TABLE 4

Table 4 gives the data collected in the case of Dr. M. D., who is 5 feet 9½ inches in height and weighs 190 pounds. His tidal air amounts to about 500 cc., and his complemental air to 1200 cc. When breathing in 2200 cc. following a forced expiration, his lungs would contain about 500 cc. more than at the end of a normal inspiration, or when breathing 3600 cc. as above, 1800 cc. more than is present at the end of a normal inspiration. His vital capacity was about 3600 cc.

EXPERIMENT NUMBER	AIR INSPIRED FROM BAG A	AIR EXPIRED INTO SPIROMETER B	CO <sub>2</sub> IN SPIROMETER B	AMOUNT CO <sub>2</sub> IN SPIROMETER B
	cc.	cc.	per cent	
1	2200	1000	3.55	35.5
2	2200	1000	3.50	35.0
3	2200	790	3.40	26.8
4	2200	700	3.30	23.1
5	2200	650	3.25	21.2
6	2200	400	2.75	11.0
1	3600	1500	3.00	40.5
2	3600	1050	2.90	30.4
3	3600	800	2.75	22.0
4	3600	575	2.40	13.8

When 2200 cc. of air are inspired:

- By combining 1 and 6 we find 130 cc. dead air space
  - By combining 1 and 5 we find 132 cc. dead air space
  - By combining 1 and 4 we find 140 cc. dead air space
  - By combining 6 and 3 we find 121 cc. dead air space
  - By combining 6 and 4 we find 128 cc. dead air space
- Average dead air space when 2200 cc. are inspired is 131 cc.

When 3600 cc. of air are inspired:

- By combining 1 and 4 we find 185 cc. dead air space
  - By combining 1 and 3 we find 182 cc. dead air space
  - By combining 1 and 2 we find 195 cc. dead air space
  - By combining 2 and 4 we find 182 cc. dead air space
  - By combining 3 and 4 we find 196 cc. dead air space
- Average dead air space when 3600 cc. are inspired is 184 cc.

#### Summary

- 2200 cc. inspired gives a dead air space of 131 cc.
- 3600 cc. inspired gives a dead air space of 184 cc.

is most important to note that variations in the dead air space in the ordinary volumes of respiration are slight, and this point makes it possible to determine the percentage composition of the alveolar air with reasonable accuracy by the examination of the gaseous content of the expired air, correction being made for the dead air space. It is also interesting to note that the volume of the respiratory dead air space thus determined is somewhat smaller than has hitherto been thought.

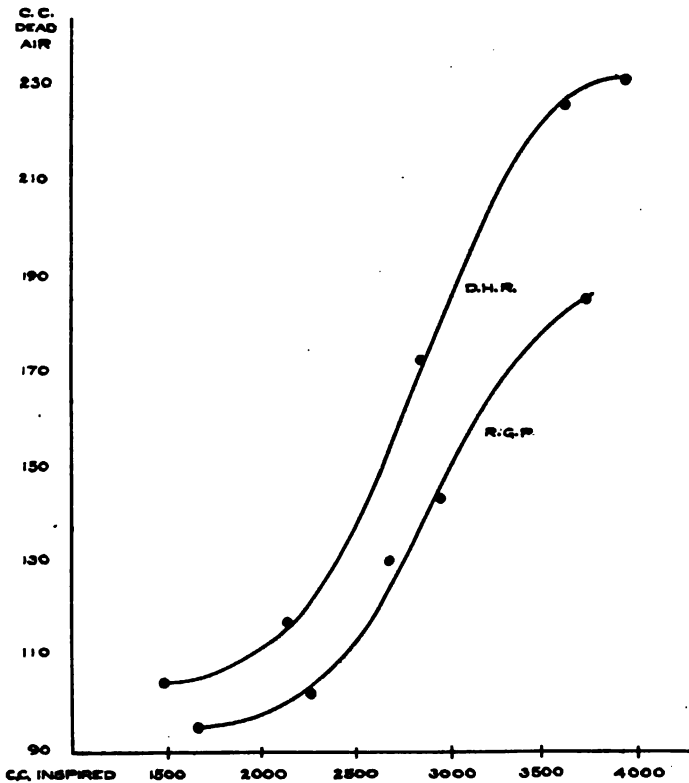


FIG. 2

It was hoped that evidence might be obtained of normal variations in the respiratory dead air space at different times of the day and under varying conditions. Such variation has been noted previously by us when the dead air space was determined by the method described in previous articles of this series. The experimental error of the method is probably too great to allow this to be done.

## SUMMARY

1. A new method of determining the capacity of the dead air space is described.
2. The volume of the respiratory dead air space is relatively constant at all volumes of respiration.
3. The changes which occur in the volume of the dead air space when the variations in depth of breathing are such as ordinarily occur during moderate effort, are not of as great magnitude as when forced respiratory efforts are made.
4. The percentage composition of the alveolar air can be determined with approximate accuracy from analysis of the expired air and an assumed volume of the dead air space, if the depth of breathing is not excessive. This method is as reliable as the best direct methods now used for the determination of the percentage composition of alveolar air, providing the respiratory volume is large enough to completely wash out the dead air space.

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# THE SUB-ARACHNOID AND INTRA-ARTERIAL ADMINISTRATION OF SODIUM BICARBONATE AND OTHER ELECTROLYTES

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## INTRODUCTION

It has been shown that extensive voluntary hyperpnoea may produce mild tetany in the normal human subject (1). The causation of this effect was interpreted as a mild alkalosis due to the "washing out" of  $\text{CO}_2$  from the blood at the lung surface. A certain amount of evidence in support of this view is given by the fact that on two occasions mild tetany was produced in dogs by large injections of sodium bicarbonate. It would therefore appear that such tetany is due either to a decrease in the  $\text{C}_x$  of the blood or else to a disturbance in the sodium, potassium, calcium equilibrium within the nervous tissue. In order to throw further light upon this subject a number of experiments were carried out on dogs, rabbits, frogs, and turtles, in which the effects of sub-arachnoid and intra-arterial injections of various electrolytes were studied. It has been shown by Weed and Wegeforth (2) that irrigation of the spinal and cerebral sub-arachnoid spaces with bicarbonate-free Ringer's solution can be carried out without serious effect, whereas irrigation with isotonic solutions of sodium chloride results in the manifestation of severe toxic effects. They found also, when the irrigation fluid was free from KCl that no noticeable toxic symptoms developed, while irrigation with an isotonic Ca-free solution resulted in the same toxic manifestations as when 0.7 per cent to 1 per cent NaCl in distilled water was used. The importance of the Ca ion in relation to physiological activity has frequently been emphasized (3). Locke (4) showed that the Ca ion is necessary for the transference of the excitory process from nerve to muscle and Overton (5) demonstrated that it is equally necessary for the transmission of the excitory

state through the synapse. It is very probable that a variety of effects which may be elicited in various ways, such as will be subsequently described, are in a large measure due to a disturbance in the Ca balance within the nerve cell.

#### METHODS

The effects of sub-arachnoid injections of various electrolytes were studied in both normal and anesthetised animals. Dogs were used for the most part but experiments were also carried out on rabbits, frogs, and turtles. The animal, in practically all experiments where graphic records were taken, was first placed under morphine-ether anesthesia, a cannula was then inserted in the left carotid artery and connected to the mercury manometer, tracheotomy was performed and anesthesia maintained by the ether bottle method described by Jackson (6). The respiratory movements were recorded by means of a Marey tambour which was connected by rubber tubing to a glass cannula which was inserted into the rubber tubing connecting the tracheal cannula with the valved top of the ether bottle. The valves in the ether bottle were so adjusted that uniform anesthesia was maintained, the tracing of the Marey tambour lever then being a relative index of the rate and depth of the respiratory movements. Two needles were, as a rule, placed in the spinal canal, one in the lumbar, the other in the subcerebellar region.

Injections into the sub-arachnoid space were made very slowly and cautiously and the volume of fluid injected at any one time was small as the records indicate. As a rule 2 or 3 cc. of spinal fluid (in large dogs) was drawn off before an injection was made or else the obturator was removed from the needle for two or three minutes prior to making the injection, and this latter was not replaced until a few minutes after the injection had been made. These precautions were taken in order to exclude the possibility of any effects elicited by spinal injection being due to increased pressure of the cerebro-spinal fluid.

Intra-cerebral injections were made through a needle placed in the third ventricle, a small opening having first been made in the parietal bone just lateral to the median plane.

Intra-venous and intra-arterial injections were also made. The external jugular vein was chosen for the former while the vertebral or carotid artery was selected for the latter.

Intra-spinal injections were made on rabbits after lumbar puncture had been performed under local anesthesia, while intra-spinal injections

were made in frogs and turtles by means of a small hypodermic needle which was placed through the occipito-atlantoid ligament. When dogs were not placed under morphine-ether anesthesia, a small amount of morphine was given by subcutaneous injection and then lumbar puncture was performed under local anesthesia.

#### RESULTS

*Sub-arachnoid and intra-arterial injections of  $\text{NaHCO}_3$ .* It was found that the injection of small amounts of sodium bicarbonate solution into the lumbar sub-arachnoid space resulted almost immediately in the manifestation of marked tetany of the musculature of practically the whole body, the muscles of the posterior half of the body being, however, more affected than those of the anterior region (protocols 1, 2 and 3). Experiments have been carried out on twenty dogs and six rabbits and in every instance where  $\text{NaHCO}_3$  has been injected into the spinal canal in appreciable amounts, tetany has resulted. Tetanic convulsions were also elicited in frogs and turtles by intra-spinal injections of mere traces of  $\text{NaHCO}_3$ . The effect can be produced in both anesthetised and unanesthetised animals. The strength of the bicarbonate solutions used varied from 1 per cent to 10 per cent. Intra-spinal injections of  $\text{NaHCO}_3$  in addition to producing tetany resulted in intensive and sometimes very much prolonged stimulation of the chief medullary centers which was manifested by a great increase in lung ventilation, rise in blood pressure and various degrees of cardiac vagus activity (fig. 1). If the  $\text{NaHCO}_3$  were injected in sufficient amount to cause most intensive tetanic spasms, respirations were inhibited and artificial respiration had to be resorted to until the spasm had modulated to a sufficient degree to make spontaneous respiration possible. It was found that tetany could be antagonized to a considerable extent by the intra-spinal injection of  $\text{CaCl}_2$  (fig. 1), while subcutaneous injection antagonized only to a slight degree. The medullary stimulation was, however, not counteracted to the same degree by the intra-spinal injection of  $\text{CaCl}_2$ . When injections of  $\text{NaHCO}_3$  were made into the sub-cerebellar cistern after occipital-atlantoid puncture, the medullary stimulation was much more marked, while tetany was less readily obtained. If but a small quantity of  $\text{NaHCO}_3$  was injected in this region there was no tetany but only violent hyperpnoea, increased blood pressure and evidence of cardiac inhibition (fig. 2). If the dose was slightly increased, in addition to the above symptoms mild

tetany of the muscles of the face, neck, shoulders, and fore limbs was produced, while a still larger injection caused the tetany to become general. Intra-cerebral injection of  $\text{NaHCO}_3$  through a needle placed in the third ventricle produced somewhat similar effects as injection into the subcerebellar cistern (fig. 3, e). While intra-venous injections of comparatively large quantities of  $\text{NaHCO}_3$  were without marked effect it could be shown that the medullary stimulation following the sub-arachnoid injection of  $\text{NaHCO}_3$  was due in part at least to the direct

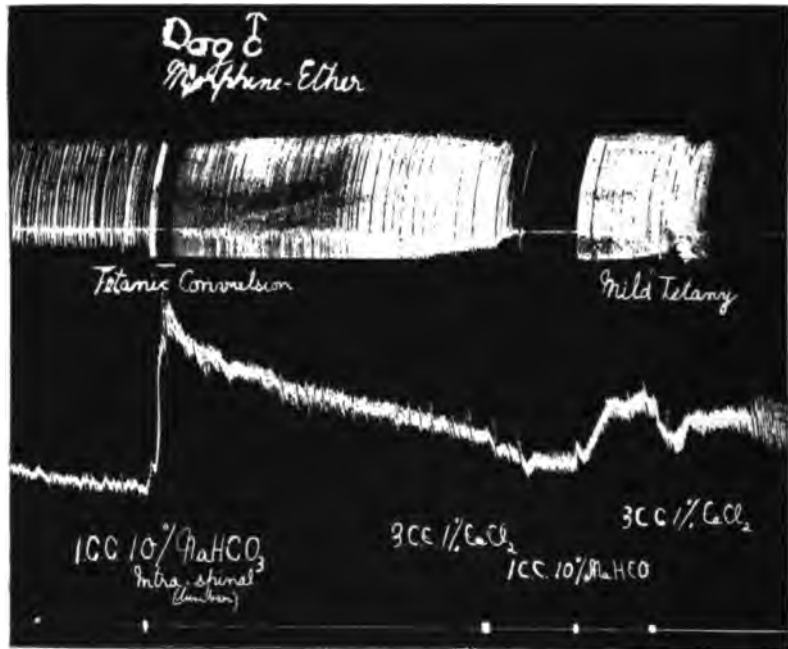


Fig. 1. Tetanic convulsions following intra-spinal injection of  $\text{NaHCO}_3$ . Effect antagonized by  $\text{CaCl}_2$ . Time, 3-second intervals.

action of the latter upon the nerve cells of the medulla. This was demonstrated in the following manner. A cannula was placed in the left carotid artery and connected with a mercury manometer. The right vertebral and carotid arteries were then dissected free and  $\text{NaHCO}_3$  was injected at different periods into both of these vessels. In other instances injections were made into the central end of the ligated carotid artery. It was found that intra-arterial injection of this type resulted at once in stimulation of the medullary centers.

The immediate effect of simultaneous stimulation of the respiratory, vasomotor and cardio-inhibitory centers by  $\text{NaHCO}_3$  varied in different animals. The first effect was in some cases complete cardiac inhibition marked by a great fall in blood pressure (fig. 2). This however passed off in a few seconds, and the blood pressure rose to a higher point than it was formerly. That the cardiac inhibition just mentioned was due to strong stimulation of the cardio-inhibitory center was shown

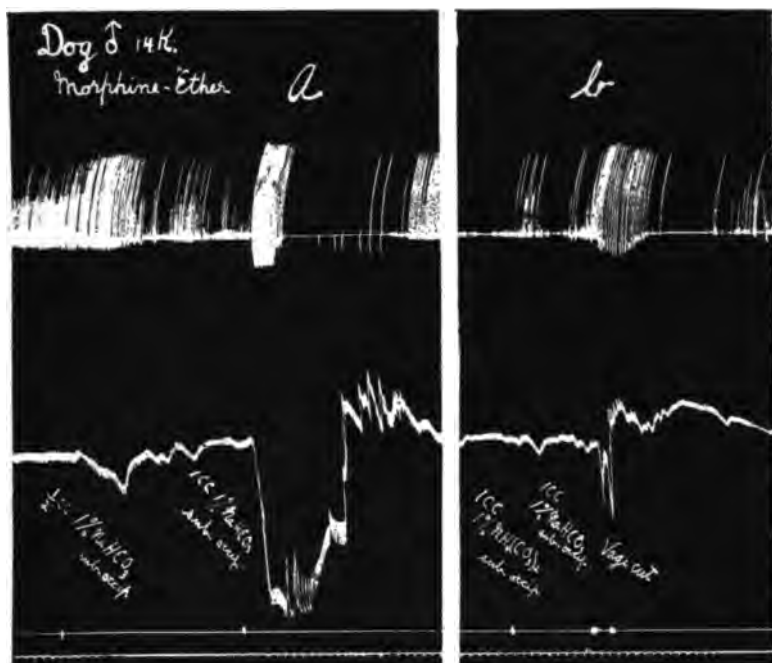


Fig. 2. *a.* Vagus inhibition following injection of  $\text{NaHCO}_3$  into cisterna magna. *b.* Same after 4 minutes. Effect of  $(\text{NH}_4)_2\text{CO}_3$  and of  $\text{NaHCO}_3$ . Vagi cut immediately after injection of  $\text{NaHCO}_3$ . Time, 3-second intervals.

by cutting both vagi. When the vagi were cut sub-arachnoid injection of  $\text{NaHCO}_3$  resulted in an immediate and extensive rise in blood pressure. As has been stated previously, slight tetany was obtained in dogs on two occasions after large intra-venous injections of  $\text{NaHCO}_3$ ; this observation was the exception, however, and not the rule. It has also been demonstrated that occasionally one may obtain increased lung ventilation in dogs under ether anesthesia following intra-venous in-

jection of sodium bicarbonate. Under morphine-ether, however, the usual effect of  $\text{NaHCO}_3$  given by intra-venous injection is a decrease in respiratory activity.

*Sub-arachnoid injections of Locke's solution.* It was found that sub-arachnoid and intra-cerebral injection of normal Locke's solution was without marked effect, while injection of concentrated Locke's solution caused definite stimulation of the medullary centers.

*Sub-arachnoid injections of distilled water.* Sub-arachnoid injection of 2 cc. of distilled water in the lumbar region of an unanesthetised rabbit caused after a latent period of one and one-half minute a tetanic convulsion which lasted for a period of seven minutes. Artificial respiration was performed during this period. Spontaneous respirations were resumed with the relaxation of the muscular spasm and an increase both in the amplitude and the rate of the respiratory movements was noted (protocol 4). During the next twenty minutes the tetany gradually subsided, the animal gaining control of the muscles of the anterior half of the body first, while at the end of half an hour it was able to run about. Sub-arachnoid injection of distilled water into the lumbar region of anesthetised dogs was followed by a rise in blood pressure, greatly increased lung ventilation and mild tetany of the muscles of the posterior half of the body. Injection of distilled water into the sub-cerebellar cistern caused stimulation of the medullary centers. Rapid injection of distilled water into the vertebral or carotid artery caused slight increase in blood pressure and very mild stimulation of the respiratory center (fig. 4).

*Sub-arachnoid injections of sodium chloride.* Hypertonic solutions of  $\text{NaCl}$  injected into the sub-arachnoid space of the lumbar region of rabbits produced practically the same type of effect as  $\text{NaHCO}_3$  or distilled water (protocol 5). Injection of hypertonic  $\text{NaCl}$  solution into the lumbar region of the spinal canal of anesthetised dogs caused a mild degree of tetany, increased respiratory movements and a rise in blood pressure. The tetany was antagonized by  $\text{CaCl}_2$ ; Weed and Wegeforth (2) have previously observed toxic effects during irrigation of the spinal canal with isotonic  $\text{NaCl}$ . Injections of hypertonic  $\text{NaCl}$  solution into the sub-cerebellar cistern resulted in strong stimulation of the medullary centers. If too great a quantity were injected these centers were paralyzed but could be again activated if  $\text{CaCl}_2$  were injected at once (fig. 3). The injection of hypertonic  $\text{NaCl}$  into the vertebral or carotid artery caused slight stimulation of the medullary centers.

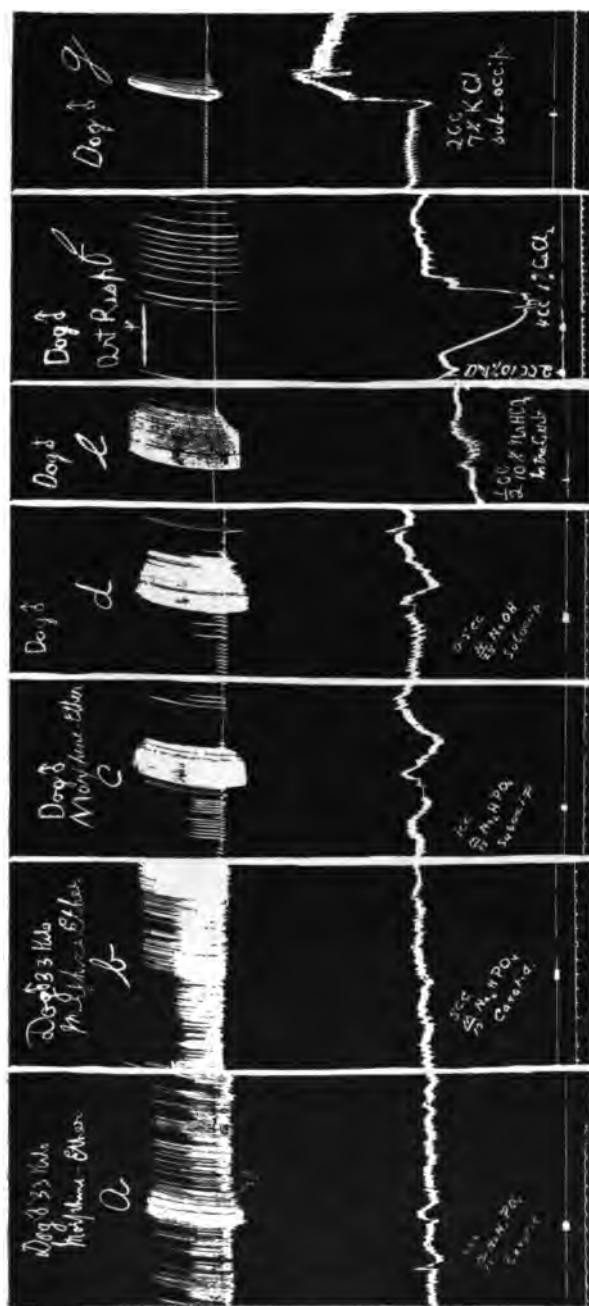


Fig. 3. a. Effect of injection of 6 cc. of  $\frac{M}{10}$   $\text{NaH}_2\text{PO}_4$  into carotid artery. b. Effect of injection of 5 cc. of  $\frac{M}{10}$   $\text{Na}_2\text{HPO}_4$  into carotid artery. c. Effect of injection of 1 cc. of  $\frac{M}{10}$   $\text{Na}_2\text{HPO}_4$  into cisterna magna. d. Effect of injection of  $\frac{1}{4}$  cc. of  $\frac{M}{10}$   $\text{NaOH}$  into cisterna magna. e. Effect of intra-cerebral injection of  $\frac{1}{4}$  cc. of 10 per cent  $\text{NaHCO}_3$ . f. Demonstration of antagonistic action of  $\text{NaCl}$  and  $\text{CaCl}_2$ . g. Effect of injection of 2 cc. of 7 per cent  $\text{KCl}$  into cisterna magna. Time, 1-second intervals.

*Sub-arachnoid injections of KCl.* Hypertonic solutions of KCl injected into the sub-arachnoid space or into the carotid or vertebral artery were found to have the same effect as hypertonic solutions of NaCl, but to a much greater degree (fig. 3, *g*).  $\text{CaCl}_2$  antagonized the action of KCl in much the same manner as it did NaCl.

*Sub-arachnoid injections of  $(\text{NH}_4)_2\text{CO}_3$ ,  $\text{MgSO}_4$ ,  $\text{Na}_2\text{HPO}_4$  and  $\text{NaH}_2\text{PO}_4$ .*  $(\text{NH}_4)_2\text{CO}_3$  was found to have a mild stimulating effect on



Fig. 4. Effect of injection into carotid artery of  $\frac{N}{16}$  NaOH,  $\frac{M}{16}$  neutral phosphate, distilled water and  $\frac{M}{16}$  acid phosphate. Time, 10-second intervals.

the medulla (fig. 2), while  $\text{MgSO}_4$ , as has been well established, had a depressant action. Both basic and acid phosphates injected into the subcerebellar cistern caused increased lung ventilation while neutral phosphate had a slight stimulating action. Intra-arterial injection of both acid, basic and neutral phosphate solutions caused increased respiratory activity (figs. 3 *a*, and 4). Intra-spinal injection of basic phosphate produced tetany in the rabbit (protocol 6).



*Sub-arachnoid injections of NaOH.* One cubic centimeter of  $\frac{N}{16}$  NaOH injected into the lumbar sub-arachnoid space of a rabbit caused mild tetany in all muscles of the body which was followed by a temporary paralysis of the muscles of the lower extremities (protocol 7). Three cubic centimeters N. sodium hydroxide injected into the lumbar region of the spinal canal of an 8-kilo dog under the influence of morphine caused increased tonus of all muscles. Two cubic centimeters of 10 per cent  $\text{NaHCO}_3$  injected into the same region after an interval of twenty minutes caused intensive tetanic spasms of the whole musculature. Three cubic centimeters of  $\frac{N}{16}$  NaOH injected into the lumbar region of a dog under morphine-ether anesthesia produced very little effect while 1 cc. of  $\frac{N}{16}$  NaOH injected into the sub-cerebellar region of another animal caused a decrease in respiratory movement and a slight fall in blood pressure. One-half cubic centimeter of  $\frac{N}{16}$  NaOH placed in the sub-cerebellar cistern caused in another instance decreased lung ventilation and stimulation of the cardio-inhibitory and vasomotor centers. Injection of 10 cc. of  $\frac{N}{16}$  NaOH into the carotid artery of one animal caused definite stimulation of the respiratory center (fig. 4).

*Irrigation of the spinal canal.* Irrigation of the spinal canal with isotonic Ringer-Locke's produced little effect, while irrigation of the sub-arachnoid spaces with Ringer-Locke's containing 0.3 per cent  $\text{NaHCO}_3$  produced intense hyperpnoea. It was found when the cerebrospinal fluid was washed out from the sub-arachnoid spaces and replaced by isotonic saline that, after a period of half an hour had elapsed, it was practically impossible to secure fluid from the sub-cerebellar cistern. This was attributed to the inability of the practically protein-free fluid to resist absorption from the sub-arachnoid area.

The protocols which follow indicate in greater detail the manner in which some of the experiments referred to above have been carried out.

#### PROTOCOLS

##### 1. Rabbit ♂, weight 2 kilos

- 3:55 p.m. Cocaine hydrochloride 0.5 grain subcutaneous and intra-muscular over the fourth lumbar vertebra.
- 4:00 p.m. Injection of 2 cc. 10 per cent  $\text{NaHCO}_3$  in distilled water into the sub-arachnoid space of the lumbar region.
- 4:01 p.m. Tetanic convulsion onset of which caused the animal to turn a complete somersault.

- 4:01 to 4:30 p.m. Gradual diminution of the tetany with hyperpnoea throughout.  
 4:45 p.m. Animal able to run around.  
 Animal died the following day.

*2. Dog ♀ 6.5 kilos*

- 3:45 p.m. 1 gr. morphine subcutaneous.  
 4:10 p.m. 0.5 gr. of cocaine subcutaneous and intra-muscular over fourth lumbar vertebra.  
 4:17 p.m. 4 cc. of 5 per cent  $\text{NaHCO}_3$  injected into the lumbar sub-arachnoid space.  
 4:17½ p.m. Tetanic spasm of the entire body but most marked in the posterior half.  
 4:30 p.m. Tetany practically gone.  
 4:50 p.m. 2 cc. of 1 per cent  $\text{CaCl}_2$  injected into the lumbar sub-arachnoid space.  
 5:00 p.m. 5 cc. of 5 per cent  $\text{NaHCO}_3$  injected without effect.

*3. Dog ♂, 40 kilos*

- 10:25 a.m. 4 gr. morphine subcutaneous.  
 11:00 a.m. Ether anesthesia.  
 11:15 a.m. Tracheotomy and cannula in left carotid connected for recording blood pressure.  
 11:40 a.m. Lumbar puncture. Clear fluid from needle.  
 11:45 a.m. 1 cc. of 10 per cent  $\text{NaHCO}_3$  injected through needle in lumbar sub-arachnoid space.  
 Tetany confined to posterior half of body developed at once, rate and depth of respirations increased, and increase in blood pressure from 120 mm. to 200 mm. Hg.  
 11:55 a.m. 1 cc. of  $\text{CaCl}_2$  by lumbar needle.  
 11:58 a.m. 1 cc. of 10 per cent  $\text{NaHCO}_3$  by lumbar needle. No tetany. Hyperpnoea and rise in blood pressure from 130 mm. to 165 mm. Hg.

*4. Rabbit ♂, 2 kilos*

- 12:00 noon. 0.5 gr. cocaine hydrochloride sub-cutaneous and intra-muscular over fourth lumbar vertebra.  
 12:05 p.m. Lumbar puncture.  
 12:06 p.m. 2 cc. distilled water slowly injected into spinal canal.  
 12:07½ p.m. Intense tetanic spasm of all muscles.  
 12:10 to 12:13 p.m. Artificial respiration.  
 12:13 to 12:45 p.m. Tetany diminished gradually.  
 12:45 p.m. Animal has control of fore-limbs, head and shoulders.  
 12:55 p.m. Animal able to run about.  
 Next day. Completely recovered.

*5. Rabbit ♂, 1.5 kilo*

- 4:00 p.m. 0.5 gr. cocaine hydrochloride sub-cutaneous and intra-muscular over fourth lumbar vertebra.  
4:05 p.m. Lumbar puncture.  
4:07 p.m. 2 cc. of 10 per cent NaCl intra-spinal.  
4:08 p.m. Tetany of whole musculature.  
4:13 p.m. Tetany considerably diminished.  
4:30 p.m. Animal running about.  
Next day. Animal normal.

*6. Rabbit ♂, 2 kilos*

- 4:00 p.m. 0.5 gr. cocaine hydrochloride sub-cutaneous and intra-muscular over fourth lumbar vertebra.  
4:05 p.m. 0.5 cc.  $\frac{M}{15}$   $\text{Na}_2\text{HPO}_4$  intra-theal.  
4:05½ p.m. Tetanic spasm most marked in posterior extremities.  
4:10 p.m. Tetany practically gone.  
4:15 p.m. Animal running about.  
4:20 p.m. 0.5 cc. of  $\text{NaH}_2\text{PO}_4$  intra-theal; no effect.

*7. Rabbit ♂, 2 kilos*

- 4:00 p.m. 0.5 gr. cocaine hydrochloride sub-cutaneous and intra-muscular over the fourth lumbar vertebra.  
4:02 p.m. Lumbar puncture.  
4:05 p.m. 0.5 cc. of  $\frac{N}{10}$  NaOH intraspinal.  
4:05½ p.m. Mild tetany of whole musculature.  
4:15 p.m. Tetany practically gone but animal has no control of lower extremities.  
5:00 p.m. Only partial control of hind legs.  
Next day: Slight paralysis of posterior extremities.  
Five days later: Complete recovery.

## DISCUSSION

It was thought at first that the tetany following intra-spinal injection of  $\text{NaHCO}_3$  was due in part to the change in the  $\text{C}_\pi$  of the nerve cells as a result of increased concentration of bicarbonate and in part to the specific action of the  $\text{HCO}_3$  ion. When, however, further work disclosed the fact that similar effects could be elicited by intra-spinal injections of such substances as distilled water, NaCl, KCl and NaOH, it became apparent that the factors of H and  $\text{HCO}_3$  ion concentration were probably of secondary importance. The change in concentration of Ca ion within the nerve cell either relatively or absolutely in relation to the

concentration of Na and K ions would seem to be the direct cause of the symptoms manifested in these experiments. As the effect of  $\text{NaHCO}_3$  is relatively greater than that of the other electrolytes studied, especially of NaOH, it is possible that the bicarbonate ion as such may have a specific action. This would concur with the opinion of Macleod (7) that the respiratory center is sensitive to the  $\text{HCO}_3$  ion. It is assumed, therefore, that change in the relative as well as absolute concentrations of the various kations is the chief underlying cause for the various phenomena noted in the experiments which have been quoted. Disturbance of the concentration of the Ca ion is, however, the chief factor. As physiologically balanced saline solutions are practically without effect while distilled water and solutions of Ca-free electrolytes in various concentrations produce such marked symptoms, it is evident that in all these latter instances the concentration of the Ca within the nerve cell must have been reduced as a result of the diffusion of the same out of the cell due to the difference in concentration established as a result of the injection. The administration of weak alkaline solutions such as NaOH,  $\text{NaHCO}_3$  and  $\text{Na}_2\text{HPO}_4$  would cause as well an actual fixation of a certain amount of Ca within the tissue, the concentration of Ca ion thereby being appreciably diminished.

The fact that sub-arachnoid injection of sodium bicarbonate caused effects which in magnitude are out of all proportion to the somewhat similar effects produced by NaOH would tend to show that the  $\text{HCO}_3$  ion or the  $\text{NaHCO}_3$  molecule has a specific stimulatory action. Large amounts of  $\text{NaHCO}_3$  intra-spinal are definitely toxic, and result in paralysis of the motor nerve cells. It is also true that paralysis of the respiratory center results after large injections of distilled water or almost any neutral electrolyte. It is possible that the phosphate ion may also have a specific stimulatory action on the medulla as both the acid and basic salts produce somewhat similar effects on the medullary centers. Intra-spinal injections into the lumbar region, however, disclosed the fact that tetany is readily produced by the basic but not by the acid salt. This, taken in conjunction with the medullary stimulation by acid, basic and neutral phosphate solutions suggests a certain amount of specific action of the phosphate ion on the medulla.

The tetany which is produced by lumbar sub-arachnoid injection of  $\text{NaHCO}_3$ , distilled water, hypertonic solutions of NaCl and other electrolytes, is due in the main to direct stimulation of the motor cells in the anterior horn. One cannot exclude, however, the possibility of the stimulation of the anterior horn cells as a result of irritation of the

afferent fibers in the posterior roots or the intercalated neurones in the spinal reflex arcs. The stimulation of the respiratory and vasomotor center must under the above circumstances be largely reflex as the effect is manifested before the injected fluid can possibly reach the medullary centers. When, however, one makes the injection into the sub-cerebellar cistern there is, no doubt, direct stimulation of the nerve cells in the medulla. The fact that cardiac inhibition through the vagi is produced much more readily by sub-cerebellar injection than by lumbar injection is very significant in this respect. The cardio-inhibitory center is not stimulated reflexly to the same degree as are the vasomotor and respiratory centers, while direct stimulation of the medullary centers results in a very definite cardiac inhibition, which is manifested by a marked fall in blood pressure. This effect then gives way and the blood pressure rises due to general vasoconstriction.

The experiments which are quoted in this paper demonstrate very clearly that substances in solution administered by the sub-arachnoid channel act in a very short space of time directly on the nerve cells of the brain and cord. While the effects following sub-arachnoid injections are, for obvious reasons, much more pronounced than those following injections into the carotid or vertebral artery, yet the similarity of the symptoms manifested after arterial and sub-cerebellar injection of  $\text{NaHCO}_3$ , for example, are very significant. The very definite stimulation of the respiratory and vasomotor centers following intra-arterial injection of a potentially strong alkali like 10 per cent  $\text{NaHCO}_3$  is positive proof of the sensitivity of these centers to the  $\text{HCO}_3$  ion irrespective of blood  $\text{C}_{\text{H}}$ . The decrease in lung ventilation which follows, as a rule, slow and continuous intra-venous injections of  $\text{NaHCO}_3$ , is probably due to the lowering of the hydrogen ion concentration of the blood, without there being a concomitant increase of sufficient magnitude in the bicarbonate ion to neutralize or to overcome the effect of the latter. Definite stimulation of the respiratory center can, however, be occasionally obtained during intra-venous injection of  $\text{NaHCO}_3$ . It is of interest in this respect that such an effect is more likely to be manifested by an animal under ether rather than under morphine-ether anesthesia. When massive doses of  $\text{NaHCO}_3$  are given comparatively rapidly by intra-venous injection, it is possible to obtain sudden collapse of the heart without paralysis of the respiratory center. In one instance spontaneous respiratory movements continued for two minutes after the heart had failed completely.

While it is possible to obtain direct action of a substance on the nerve cells by sub-arachnoid injection, the passage of such substance into the general circulation may take place very slowly or in some instances it may not be removed from the spinal canal at all (8).

The demonstration of Wilson (9) that parathyroid tetany is associated with an alkalosis of the blood, and the findings of MacCallum, Kellogg and Voegtlin (10) that symptoms like those of tetany can be induced by deficiency of calcium and symptoms following parathyroidectomy relieved by calcium administration would be amplified by the experiments herein recorded.

#### CONCLUSIONS

It would seem logical to conclude that the tonus of effector nerve cells and probably of all nerve cells is regulated not only by the  $C_{\pi}$  of the blood and spinal fluid but also by the maintenance in them of a definite equilibrium between the various ions. Of these latter the Ca ion is preëminent. Decrease in Ca ion leads to increased tonus, and increase in the Ca ion to decreased tonus. Slight increase in the concentration of either Na or K is marked by definite stimulation of the medullary centers while the stimulatory effect of the bicarbonate ion is very definite. The importance of Na, K and Ca ions as regards regulation of the respiratory center has been suggested by Howell (11).

It would seem probable that one of the underlying causes of tetany, as observed clinically, is a disturbance in the kation equilibrium within the nerve cells of the brain and cord.

Results, such as are herein reported, serve to emphasize the importance of definite ions of electrolytes in definite concentration within the living cell, a principle which has never been lost sight of since the pioneer experiments of Sidney Ringer (12) were published.

In conclusion I wish to acknowledge the assistance of Dr. P. L. Backus in the experimental work reported in this paper.

#### SUMMARY

1. Sub-arachnoid injection on the lumbar region of  $\text{NaHCO}_3$ ,  $\text{NaCl}$  and distilled water is followed by violent tetany and definite stimulation of the medullary centers.
2.  $\text{CaCl}_2$  administered by intra-spinal injection antagonizes tetany so produced.

3. Injections of  $\text{NaHCO}_3$ ,  $\text{NaCl}$  and  $\text{KCl}$  into the sub-cerebellar cistern cause marked stimulation of the respiratory, vasomotor and cardio-inhibitory centers.  $\text{CaCl}_2$  antagonizes this effect to a limited extent.

4. Injection into the carotid or vertebral artery of  $\text{NaHCO}_3$ ,  $\text{NaCl}$  or  $\text{KCl}$  caused definite stimulation of the respiratory and vasomotor centers.

5. Intra-venous injection of  $\text{NaHCO}_3$  may cause increased lung ventilation.

6. It is held that the bicarbonate ion has a specific stimulatory effect upon the nerve cells of the medulla and the motor cells of the anterior horn.

7. Disturbance in the concentration of the various kations particularly of the  $\text{Ca}$  ion within the nervous tissue produces most marked effects.

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## THE INFLUENCE OF INTERNAL SECRETIONS ON BLOOD PRESSURE AND THE FORMATION OF BILE

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In connection with work previously reported (1) on the influence of internal secretions on the formation of bile, a study of the blood pressure was made. The object of this was twofold: to observe the effect on blood pressure of the particular gland substance being studied, and to determine what relation, if any, existed between blood pressure changes and the amount of bile secreted after a gland substance had been administered intravenously. The following gland substances were employed: mammary, orchic, ovarian, pancreatic, splenic, thymic and thyroid. These were all obtained from Armour & Company. To this list was added solution of adrenalin chloride prepared by Parke, Davis & Company, and secretin prepared by a method described in connection with other experiments (2). These were all given by intravenous injection. Nearly all of the experiments were performed on dogs but occasionally cats were used. A description of the technique will be found in the article to which reference has been made (1) and the numbers of experiments mentioned in this contribution correspond to the experiment numbers there given. All observations were made for arbitrary periods of twenty minutes each. The usual procedure was to record blood pressure and the number of drops of bile secreted during three twenty-minute periods, one immediately preceding the administration of the gland substance, a second commencing with the beginning of the injection, and a third immediately following the second. In a few cases the third record was not secured. The dose in every experiment, except those with adrenalin and secretin, was 10 mgm. of the gland substance per kilogram of body weight of the animal, dissolved in 100 cc. of physiological saline solution. This was warmed to 37°C. on a water bath and injected into the jugular vein by means of a burette. The dose of adrenalin was 0.1 cc. of a 1:1,000 solution of adrenalin chloride per kilogram of body weight, and the dose of secretin was 10 mgm. of a dried acid extract per kilogram.



*Adrenalin.* Five determinations were made with adrenalin. There was invariably an immediate rise of pressure which averaged 81.25 mm. of mercury. In the case of adrenalin the injections were made much more slowly than with the other substances studied and the maximum to which the pressure was allowed to rise was 220 mm. This high blood pressure, with slight fluctuations, was maintained for 5 minutes as an average and at the end of the 20-minute period the pressure had always fallen to or slightly below the original level.

The amount of bile secreted after the injection of adrenalin was always less than during the preceding 20 minutes and the striking feature was the marked decrease during the period of high blood pressure. For example, in experiment 13 the initial count was 140 drops distributed fairly uniformly throughout the period; during the first 5 minutes of the injection period 1 drop was secreted, in the next 5 minutes 3 drops, in the third 5 minutes 3 drops, and during the last 5 minutes 42 drops—a total of 49 drops for this 20-minute period.

The diminution produced by adrenalin in the amount of bile secreted may be explained by a decrease of both the arterial and venous inflow. As has been shown by Burton-Opitz (3), (4) adrenalin injected into the hepatic artery or portal vein exerts a local constricting action and diminishes the inflow and it is not improbable that even when the adrenalin is introduced through a vessel more distant from the liver the same action takes place.

*Mammary.* Mammary substance was used eight times and in all but one there was an initial rise in blood pressure followed by a fall and a slow return to normal. The average figures for these experiments are as follows: blood pressure at beginning of injection 109.2 mm.; maximum pressure 117.6 mm., reached 26.4 seconds after injection started; minimum pressure 95.4 mm., 71.4 seconds from beginning of injection; pressure 103.8 mm. at end of first 5 minutes, and 106.8 mm. at end of period. Experiment 46, which failed to show an initial rise in blood pressure, was similar in other respects to the experiments of the group. Ott and Scott (5) report a slight fall in blood pressure for a few seconds after intravenous injection of mammary substance but there is no record of the preliminary rise which occurred so uniformly in our experiments.

So far as bile production was concerned the effect of mammary gland substance was not uniform but it would seem to be a temporary decrease. In experiment 11 the falling off in the rate of secretion was most marked immediately after the injection. Twenty-three drops of

bile were secreted during the twenty-minute period following the injection as compared with an initial count of 46; 14 of the 23 drops came during the last half of the period whereas the original 46 drops had been quite uniformly distributed. Experiment 8 was similar; the initial count was 3 drops, but after the injection there was no secretion until the last 4 minutes of the period when 2 drops were recorded. On the other hand, experiments 16, 46 and 57 showed a progressive decrease in the formation of bile. The other experiments of the group showed no change or an increased production.

From these records it appears that not only is mammary substance inconstant in its effect on the secretion of bile but that there is no relation between changes in blood pressure and bile formation under its influence.

*Orchic.* Seven experiments were made with orchic substance. Four showed an initial rise of pressure followed by a fall slightly below normal, a gradual return to the normal level and the pressure at the end of the period approximately the same as at the beginning; two did not show an initial rise, but a slight fall and then a gradual rise above the original level persisting to the end of the period. In one, experiment 4, no blood pressure record was made. The average figures for the first group of experiments, viz., 18, 41, 42 and 55, are as follows: blood pressure at beginning of injection, 128 mm. of mercury; maximum pressure 147.2 mm., reached 62.5 seconds from beginning of injection; minimum pressure 124 mm. at 110.7 seconds after injection commenced; pressure at end of first 5 minutes, 134.7 mm., and at end of period, 126.5 mm. For the second group of orchic experiments, numbers 19 and 62, the initial pressure is 113 mm., followed by a minimum of 99 mm. in 20 seconds, a rise to 127 mm. in 125 seconds, 123 mm. at end of first 5 minutes and 132 mm. at the end of the period.

The reported observations of the effect of the intravenous injection of testicular extract on blood pressure vary somewhat but agree in general that the pressure is lowered. In 1901 Dixon (6) described an immediate and considerable transient fall of blood pressure accompanied by cardiac slowing following the injection of orchitic extract. Vincent and Sheen (7) and Miller and Miller (8) note the production of a fall in pressure after testicular extract. Vincent (9) and Ott and Scott (5) state that the fall in pressure is slight and lasts for a few seconds only. Wheelon (10) records a slight fall in blood pressure after castration. Bingel and Strauss (11) were unable to produce a change in pressure by the administration of extract of testis.

Orchic gland substance caused a decrease in the amount of bile formed in five of the seven experiments and in four of these the effect was progressive, a further decrease occurring in the second 20-minute period after the injection. One interesting feature presents itself in an analysis of these records: there was a primary decrease during the first 15 minutes following the injection, a rise during the next 15 minutes, and a second decline beginning about 30 minutes after the gland substance was administered. This second depression of secretory activity was more marked than the first.

A comparison of blood pressure changes with variations in the amount of bile secreted fails to show any uniformity. Of the four experiments showing a rise in blood pressure, then a fall and a gradual return to normal, three gave a decrease in bile formation and one, number 55, an increase of 108.33 per cent. In the two experiments where there was a fall of pressure followed by a rise to a point 18 mm. above the original height at the end of the period, the changes in pressure were accompanied by decreased production of bile in one, experiment 19, and increased production in the other, experiment 62.

*Ovary.* Ovarian substance was used five times, in four of which blood pressure records were made. Two cases, experiments 20 and 59, showed a rise of pressure shortly after the injection began, then a fall below normal, a second rise above the original level, and a gradual return to normal. Averages: initial pressure, 130 mm. of mercury; first maximum, 148.5 mm., 19.5 seconds after injection began; minimum 119.5 mm., 44 seconds from beginning; second maximum 137.5 mm. at end of first 3 minutes, and final pressure 132.5 mm. Two other experiments, 43 and 56, with ovarian substance failed to show changes similar to the above, the pressure remaining almost constant. In experiment 5 no blood pressure record was secured.

The effect of ovarian gland substance or extract of ovary on blood pressure has been reported by Ott and Scott (5), Vincent and Sheen (7), Miller and Miller (8), Vincent (9) and Goncalons (12). These investigators agree that the effect is a lowering of blood pressure which is usually slight and transient.

Ovarian substance invariably produced a decrease in the amount of bile formed. The average decrease was 44.59 per cent in the first period and 59.36 per cent in the second period after the injection.

*Pancreas.* This substance was employed in nine experiments and for six of these complete blood pressure records are available. The effects on blood pressure fall into two groups, one showing a prompt

and very marked fall in blood pressure, the other showing a preliminary slight rise followed by a fall. In all of these records there is a rather characteristic lowering of blood pressure which is still in evidence at the end of the period, but from which there is gradual recovery during the second 20-minute interval. Forty minutes after injection the blood pressure averaged 121 mm. as compared with 123.6 mm. immediately preceding the injection.

The first group consists of four experiments, 22, 38, 48, 50, and shows the following: initial pressure 123.2 mm.; fall began in 5.5 seconds and the minimum pressure, 60 mm., was reached 26.7 seconds from the beginning of the injection. At the end of 5 minutes the pressure was 86.5 mm., and at the end of the period, 95.7 mm.

The second group, experiments 14 and 52, gives these averages: initial pressure, 124 mm.; 17 seconds later pressure began to rise and reached a maximum of 131.5 mm. 19 seconds after the injection was started; then fell to 77.5 mm., 40 seconds from beginning; at end of first 5 minutes was 118.5 mm., and at end of period 107 mm.

With the exception of Popielski (13), who reports a marked and prolonged rise in blood pressure as the result of the injection of an acidulated watery extract of pancreas, investigators agree that injection of the substance of the pancreas or of saline extracts of pancreas causes a fall in blood pressure. Ott and Scott (5) state that the lowering of pressure is more marked than that obtained with ovary, testis, mammary, spleen and thymus.

Biliary secretion was decreased in every case in which pancreatic substance was administered. The reduction averaged 45.45 per cent in the first period and 44.28 per cent in the second period following the injection. The striking feature in this connection was the great reduction in secretory activity immediately after the injection. The counts made during the periods preceding the injections show the drops of bile falling at a fairly uniform rate. In the first 10 minutes subsequent to the introduction of the gland substance only 19 per cent of the total secretion for the period was obtained, the other 81 per cent occurring during the second half of the period. In the next 20-minute period, the second after the injection, the rate of secretion was more uniform but still only a little more than one-half the original.

*Secretin.* Seven experiments were carried out with secretin. The blood pressure records show an average pressure preceding the injection of 114.1 mm. of mercury; 20.2 seconds after the injection was begun pressure commenced to fall and reached a low point of 56.8 mm.

40 seconds from the starting point; at the end of 5 minutes the pressure was 110.5 mm., and at the end of the period, 118.4 mm. In two experiments, 26 and 54 of this series, there was a slight rise of pressure immediately following the injection. This increase above the original pressure averaged 15 mm. of mercury and in experiment 26 was succeeded by a drop similar to that which took place in the other experiments of the group; in no. 54 there was no abrupt fall in pressure but a gradual decline with the pressure at the end of the 20-minute period nearly the same as at the beginning.

The amount of bile produced was greatly increased in every case in which secretin was employed. This increase averaged 241.52 per cent for the first 20 minutes after the injection and 413.78 per cent for the second 20 minutes.

Bayliss and Starling (14) state, "Acid extracts of the mucous membrane ( of the duodenum and jejunum) normally contain a body which causes a fall of blood pressure. This body is not secretin, and the latter may be prepared free from the depressor substance by acting on desquamated epithelial cells with acid." This has been confirmed by v. Fürth and Schwarz (15). Matsuo (16) also concludes that the depressor substance is separate from secretin, especially as acid injected into the duodenum, while producing copious pancreatic secretion, was followed by no change in blood pressure. He was, however, not able to obtain a secretin preparation which did not produce some fall in blood pressure, but the degree of the fall and the activity of the various preparations were not at all proportionate.

In our experiments it will be observed that while there was, as a rule, a fall in pressure immediately following the injection this had been recovered from in 5 minutes and from then on the pressure remained within a few millimeters of the original. At the same time the amount of bile was tremendously increased during the 40 minutes the experiments lasted.

*Spleen.* Injection of substance of the spleen was practically without effect on blood pressure. Seven trials were made and the average pressure prior to the injection was 104.4 mm.; 20 minutes after the injection it was 107.4 mm. Only two experiments showed any fluctuations that could be attributed to the injection. In experiment 21 the pressure rose from 128 mm. to 135 mm. in 20 seconds, then fell to 124 mm. 22 seconds later, rose to 136 mm. 63 seconds later, and declined gradually to 129 mm. at the end of the period. The initial pressure in experiment 61 was 130 mm. With the beginning of the injection

blood pressure commenced to fall and in 40 seconds had reached 124 mm.; from there it rose gradually to 140 mm. at the end of the period.

Ott and Scott (5) report a slight fall in blood pressure for a few seconds after the intravenous administration of splenic substance. Vincent and Sheen (7) and Vincent (9) noted the production of a transient fall in pressure by splenic extract. Oliver and Schäfer (17) state that spleen produces a preliminary fall of pressure followed by a gradual rise above normal and then a gradual return to normal. Bingel and Strauss (11) found inconstantly a temporary rise of pressure followed by a sharp fall. Miller and Miller (8) state that in their hands saline extracts of spleen invariably caused a rise in blood pressure, which was usually but not always followed by a slight fall below normal.

So far as the formation of bile after the introduction of splenic substance by vein is concerned we found no constant effect. In the first period after the injection four experiments gave a decrease, two an increase and one no change. Alterations in the rate of secretion during the second period after injection were also without harmony.

*Thymus.* Five experiments were carried out with thymic substance and no consistent effect on blood pressure noted. Experiment 39 showed a slight rise in pressure followed by a sharp fall, and a return to normal 6 minutes after the injection began.

Where any effect on blood pressure was produced by the injection intravenously of thymic gland substance, watery extracts or saline extracts of the thymus, observers are almost unanimous in reporting it as a decrease. Reference is made to Ott and Scott (5), Miller and Miller (8), Schäfer (18), Popper (19), Basch (20) and Lucien and Parisoot (21). Popielski (13) states that an acidulated watery extract of thymus caused a rise of blood pressure.

Reference to our previous paper (1) on this subject will show that we found the amount of bile secreted after the administration of thymic substance to be decreased. This effect was still in evidence in three out of four experiments in which a count was made for a second period of 20 minutes after the injection.

*Thyroid.* Substance of the thyroid gland was injected into nine dogs. In two of the experiments, 6 and 33, no record of blood pressure was obtained. The other seven all show an initial rise in pressure, a sharp fall, a second gradual rise and a fall to normal. Pressure during the second 20-minute period following the injection was practically the same as in the period preceding the injection. Average figures for these experiments follow: initial pressure 105.8 mm.; first maximum

119.4 mm., 19 seconds from beginning; minimum 86.5 mm., 43.4 seconds from beginning; second maximum 117.7 mm., 191.4 seconds from beginning, and pressure at end of period, 110.2 mm.

Other observers have usually found a fall in blood pressure to result from the administration of thyroid extract or thyroid gland substance. Oliver and Schäfer (17), Haskovec (22), Georgiewsky (23), Guinard and Martin (24), Fenyvessy (25), v. Cyon and Oswald (26), v. Fürth and Schwarz (27), all report a fall in pressure. Ott and Scott (5) used iodothylin and obtained a marked fall with subsequent gradual rise above normal. Schäfer (18) observed a considerable fall when using thyroid extract. Vincent (9) usually noted a fall but occasionally a rise of pressure. Levy (30) records no appreciable alteration in blood pressure after intravenous injection of Kendall's crystalline thyroid iodine compound. A rise in pressure is reported by Popielski (13), Heinatz (28) and Livon (29).

So far as bile formation is concerned thyroid substance was without constant effect. Experiments 6, 28, 40 and 51 show an increase averaging 82.29 per cent in the first period after the injection. Experiments 12, 17, 33, 37 and 58 show a decrease averaging 33.85 per cent for the same period. Nevertheless, in spite of the wide variations in amount of bile secreted, the blood pressure ran a very similar course in all of these experiments.

#### DISCUSSION

While the foregoing experiments are too few in number to permit definite conclusions to be drawn, one thing seems certain, viz., that there is no constant relation between blood pressure and the amount of bile secreted. Adrenalin, it is true, consistently raised blood pressure and lowered bile formation; secretin, on the other hand, where it caused a change in blood pressure, produced a lower pressure and a great increase in the flow of bile. It might be urged that thyroid gland substance owes any action it exerts upon blood pressure and bile formation to the intervention of the adrenals and this cannot be entirely controverted by our experiments. That thyroid substance increases the output of adrenalin has been shown by Bückner (31), Rudinger, Falta and Eppinger (32) and Gley and Quinquaud (33). In our experiments, however, there was no constant relationship between blood pressure and bile production after the administration of thyroid gland substance. We did not find bile production regularly decreased when blood pressure rose or vice versa. With others of the gland substances

employed the blood pressure might be lowered and bile production decreased at the same time. The most striking example of this is in the series with pancreatic substance. Intravenous administration of substance of the pancreas caused lowering of the blood pressure and lessening of the output of bile. To a lesser extent thymic substance acted in the same manner. If we place in contrast with this the effect of secretin it would seem that we are not justified in concluding that the effect on bile formation is due to the alteration in blood pressure.

With the other gland substances employed, viz., mammary, orchic, ovarian and splenic, the results were inconstant. Orchic substance, for instance, caused a rise of blood pressure in some of our experiments and a drop in others, while the production of bile was definitely lowered. After the administration of substance of the ovary the blood pressure showed oscillations, or waves of higher and lower pressure. The pressure at first rose above the original level, then fell below the initial, rose again and returned to the original. Biliary secretion was lowered in every case and an examination of the individual records of these experiments does not show any synchronism between changes in blood pressure and the rate at which the bile was secreted.

#### CONCLUSIONS

1. As a result of the experiments set forth in this paper we feel inclined to believe that some at least of the endocrine organs exert a specific influence on the secretory activity of the hepatic cells leading to the production of bile.
2. The output of bile in the dog is increased by the administration of secretin.
3. The output of bile in the dog is decreased by the administration of adrenalin, and by mammary, orchic, ovarian, pancreatic and thymic gland substances.
4. The amount of bile secreted is not affected in a constant or definite manner by the substance of the spleen and thyroid gland.
5. Blood pressure is raised by adrenalin.
6. Blood pressure is lowered by pancreatic substance and the secretin preparation employed.
7. A fall of blood pressure, ordinarily preceded by a slight rise, is caused by orchic and mammary gland substances.
8. Oscillations of blood pressure are caused by ovarian and thyroid gland substances.
9. Blood pressure is not usually affected by splenic and thymic gland substances.



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## GASTRIN STUDIES

### V. CHEMICAL STUDIES ON GASTRIN BODIES

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As a result of numerous physiological studies with extracts from duodenal and gastric mucous membranes and from many other tissues investigators, at present, are divided into two schools. One school considers pancreatic secretin on the one hand and gastrin or gastric secretin on the other hand to be different chemical substances and to act as distinctly specific secretagogues. The other school considers the substances to be identical, that they are widely distributed "vasodilators" and that they do not act as specific secretagogues. The former view is generally accepted in our modern textbooks and this is especially true as to pancreatic secretin. In a series of previous papers (1) we referred to the literature on gastric secretin and we there reported more particularly on the physiological phase of the work. Although much of the evidence there presented together with the investigations published by others leads us to believe that very likely secretin and gastrin are different substances; nevertheless, we have not been able to convince ourselves as to the correctness of either of the two views now held. In this paper we report observations which bear more particularly on the chemical phase of the work. It is evident that the isolation of these secretagogues in pure form will do more toward solving these important questions than biological studies with variously purified extracts.

#### METHODS

*Animals.* The studies reported here were controlled on dogs with Pawlow and chronic fistula stomachs. The same general procedure was followed as previously reported, that is, a collection of a one- or

two-hour spontaneous secretion before the injection of the solution and a one- or two-hour collection after the same. The volumes were noted and the free and total acidities titrated in the usual way. All the solutions used were slightly acid and were well sterilized before injecting intramuscularly.

*Extraction.* It may be recalled that Bayliss and Starling (2) attempted to meet Popielski's criticism as to the presence of "vasodilators" in secretin solutions by extracting the dried material with absolute alcohol and discarding the absolute alcohol-soluble portion. In our earlier studies we also employed this method, but later we arrived at the conclusion that we were by no means warranted to assume that the absolute alcohol did not also remove some of the gastrin activity. The studies reported here are on material which in most cases has not been thus treated with absolute alcohol and which was prepared from filtered artificial gastric juice as prepared for the manufacture of pepsin. The filtered material as obtained from the factory was coagulated by heat, filtered, and the filtrate concentrated under diminished pressure in a bath of boiling water. The thin syrup thus obtained was neutralized to faint acidity and precipitated with five to six volumes of redistilled 95 per cent alcohol. After standing for several days the solution was filtered and the filtrate again concentrated under diminished pressure with complete removal of the alcohol. The solution left, if not distinctly acid, was made so and stored under toluol in glass stoppered cylinders. It retained its activity perfectly. This concentrated solution was used as the material for various attempts at further purification and for various studies as to stability.

#### PRECIPITATION STUDIES

*Action of basic lead acetate.* The addition of basic lead acetate in excess to this partly purified solution of gastrin does not precipitate the activity as is shown by a number of experiments in which both the precipitate and filtrate were freed from lead and tested physiologically. In each case very little or no activity was found in the precipitate and most of the original activity was recovered in the filtrate. As an illustration one of three equally reliable experiments is given below.

*Dog III. Pawlow accessory stomach, January 14, 1915*

TIME	JUICE	HYDROCHLORIC ACID	
		Free	Total
	cc.	per cent	per cent
10:10 a.m. to 11:10 a.m.	1.1	0.09	0.12
11:10 a.m. ....	Injected 2 cc. Sb4B*		
12:10 p.m. ....	6.9	0.36	0.39
1:10 p.m. ....	1.4	0.36	0.40
1:10 p.m. ....	Injected 2 cc. Sb4A*		
2:10 p.m. ....	0.6	0.26	0.31
2:20 p.m. ....	Injected 2 cc. Sb4B*		
3:20 p.m. ....	2.20	0.27	0.32
4:20 p.m. ....	1.8	0.36	0.41

\* A crude extract, Sb4, was precipitated with an excess of basic lead acetate, filtered and washed. The precipitate and filtrate were decomposed to remove the lead and concentrated under diminished pressure. Both solutions were then made up to the same volume so that 2.5 cc. were equivalent to 1.5 cc. of the original Sb4. Sb4B represents the filtrate and Sb4A the lead acetate precipitate. This step in purification was frequently used in many of the other studies referred to below.

*Precipitation by picric and picrolonic acids.* Many attempts were made to precipitate the activity by picric acid in various acidities and concentrations, but in every case the activity was recovered in the filtrates. Picrolonic acid also yielded inactive precipitates. It is of particular interest to note, however, that when histamine hydrochloride is added to the same gastrin solution one obtains an active precipitate which undoubtedly is the histamine picrolonate. In these experiments the precipitate obtained was in each case suspended in dilute hydrochloric acid and shaken with benzol or amyl alcohol to completely remove the picric or picrolonic acid. The aqueous hydrochloric acid solution was then concentrated under diminished pressure and rediluted to the original volume. The filtrates from the precipitates were decomposed in the same way and also made up to the original volume after concentrating under diminished pressure. The results below are characteristic and were duplicated on other animals.

*Dog III. Pawlow accessory stomach, January 22, 1916*

TIME	JUICE	HYDROCHLORIC ACID		REMARKS
		Free	Total	
	cc.	per cent	per cent	
1st hour.....	0.7	0	Trace	Slime

Injected 1 cc. S1A (an alcoholic extract of dried crude gastrin)

1st hour after..	7.5	0.36	0.46	
2nd hour after..	3.1	0.46	0.50	

Same dog on January 27, 1916

12:30-1:30 p.m..	1.0	0	0.05	Mucin
1:30 p.m.....	Injected picrate precipitate from 1 cc. S1A			
1:30-2:30 p.m..	1.1	0	Trace	
2:30 p.m.....	Injected picrate filtrate from 1 cc. S1A			
2:30-3:00 p.m..	5.4	0.24	0.26	
3:00-3:15 p.m..	1.8	0.43	0.45	
3:15-3:30 p.m..	0.8	0.46	0.49	
3:30-4:00 p.m..	0.7	0.44	0.47	

If to 10 cc. of the same S1A solution we added 10 mgms. of histamine hydrochloride and then proceeded with the precipitation by picric acid in the same way and examined the precipitate and filtrate for activity, the following results were obtained on the same dog.

TIME	JUICE	HYDROCHLORIC ACID		REMARKS
		Free	Total	
	cc.	per cent	per cent	
1st hour.....	0.6	0	Trace	Mucin

Injected 1 cc. of the concentrated filtrate  $\approx$  1 cc. S1A

1st hour after...	7.8	0.38	0.41	
2nd hour after..	5.9	0.50	0.54	
3rd hour after..	2.3	0.46	0.51	

Injected 1 cc. from precipitate  $\approx$  1 cc. S1A plus 1 mgm. histamine hydrochloride

1st hour after...	0.7	0.27	0.28	
2nd hour after..	1.8	0.09	0.12	

The results indicate a very poor precipitation of the added histamine by the picric acid.

With picrolonic acid we have as stated before, not been able to obtain an active picrolonate precipitate from various gastrin solutions, but when we add histamine to the same solution and then precipitate in the same way with picrolonic acid we not only obtain the histamine in the precipitate but also recover the gastrin activity otherwise, just as before, in the filtrate. In conducting this experiment we first showed that we could quantitatively recover 0.010 gram histamine hydrochloride from 10 cc. water by precipitating with 50 cc. saturated aqueous picrolonic acid solution. This was shown chemically by the diazo test. Next the same amount of histamine hydrochloride was added to 10 cc. of SIA gastrin solution and precipitated in the same way by picrolonic acid. The precipitate and filtrate were decomposed in the usual way and after concentration made up to the original volume. The physiological response was as indicated in the protocol below.

*Dog III. Pawlow accessory stomach, February 9, 1916*

TIME	JUICE	HYDROCHLORIC ACID		REMARKS
		Free	Total	
	cc.	per cent	per cent	
1 hour.....	0.3	0.00	Trace	
$\frac{1}{2}$ hour.....	0.2	0.00	Trace	
Injected of the picrolonate filtrate $\approx$ 1 cc. SIA				
1st hour after...	5.8	0.37	0.43	
2nd hour after..	1.5	0.47	0.52	
Injected of the picrolonate precipitate $\approx$ 1 cc. SIA plus 1 mgm. histamine hydrochloride				
1st hour after...	5.5	0.37	0.41	
2nd hour after..	0.3	Present		

On comparing the results here with those obtained with 1 cc. of the original SIA as given in the table for January 22, we find that the response is of the same order for the picrolonate filtrate as for the original SIA. This together with the inability to precipitate gastrin by picrolonic acid shows that the added histamine was quite completely precipitated by the precipitant. This was also verified by the diazo test applied on the precipitate and filtrate. These studies certainly indicate that gastrin and histamine are not identical chemically although they both act as good secretagogues.

*Precipitation by mercuric chloride in acid solution.* After the removal of considerable foreign material by basic lead acetate and removal of the lead from the filtrate, the acid filtrate when treated with a fair excess of cold saturated aqueous mercuric chloride yields a precipitate possessing considerable activity. A second mercury precipitate could be obtained by adding an alcoholic saturated mercuric chloride solution, but both this precipitate as well as the filtrate therefrom possessed very little or no activity. There appears to be a distinct loss of activity in this mercury treatment. The protocol below illustrates the character of the findings.

*Dog III. Pawlow accessory stomach, January 29, 1915*

TIME	JUICE	HYDROCHLORIC ACID		REMARKS
		Free	Total	
	cc.	per cent	per cent	
1st hour.....	0.1	0	0	Mucus
10:50 a.m....	Injected 3 cc. Sb5D3*			
11:50 a.m....	0.1	Trace		Mucus
11:50 a.m....	Injected 1.5 cc. Sb5D2*			
12:50 p.m....	0.1	Trace		Mucus
12:50 p.m....	Injected 1.5 cc. Sb5D1*			
1:50 p.m....	3.5	0.38	0.41	
2:50 p.m....	0.9	0.38	0.47	

\* The material started with Sb5B had been treated with basic lead acetate. The first mercury precipitate, Sb5D1, after decomposition and removal of the mercury was concentrated under diminished pressure and made up to the same volume as the original Sb5B started with. The second mercury precipitate, Sb5D2, was likewise freed from mercury and after concentration made up to the same volume as the original Sb5B started with. The filtrate from the second mercury precipitate, Sb5D3, similarly treated, was made up to twice the volume of the original Sb5B.

It is to be noted that nine days previously an injection of 1 cc. Sb5B in the same dog caused a secretion of 4.0 and 1.5 cc. gastric juice during the first and second hours following the injection with acidities of 0.46 and 0.50 per cent respectively. In other words, a dose of two-thirds the size caused a better secretion than any of the fractions above. Similar experiments on other dogs and repetitions of the same experiment on other extracts confirmed these findings.

*Purine base precipitation.* To 10 cc. of a solution which had been carried through the treatment with basic lead acetate and through the mercuric chloride precipitation in acid solution, we added sodium hydrate until the solution remained only very slightly acid. We now

added 2 grams sodium acetate, 2 cc. 35 per cent sodium bisulphite solution, 4 cc. 10 per cent copper sulphate solution and boiled for 3 minutes, filtered at once and washed with boiling water. Both the precipitate and filtrate were freed from copper by hydrogen sulphide, concentrated under diminished pressure and made up to definite volume. The precipitate contained no activity but the filtrate showed good secretagogue action.

*Other precipitants.* In part of the work, as shown below, it was discovered that the gastrin activity can be extracted from an alkaline solution by amyl alcohol and that it can be recovered therefrom by shaking with dilute hydrochloric acid in water. A solution was thus prepared from material which had been treated as above; that is, with lead acetate and mercuric chloride. The hydrochloride solution thus obtained was evaporated to dryness under diminished pressure, then redissolved in water and diluted to one-fourth the original volume. The original solution referred to was already a very active one. Of this solution 1 cc. portions were taken and treated on watch glasses as indicated below. The precipitates and filtrates were separated and freed from precipitating reagent as well as possible and were then tested for physiological activity and for color reaction by the diazo benzene sulphonic acid reagent.

REAGENT AND AMOUNT	IMMEDIATE RESULT	RESULT AFTER 20 HOURS
Saturated picrolonic acid, 2 cc.	Flocculent precipitate	Same
Alcoholic zinc chloride, 1 cc.	No change	No change
Alcoholic cadmium chloride, 1 cc.	No change	Slight precipitate
Iodine in potassium iodide, 3 cc.	Heavy brown to black precipitate	No change
Potassium mercuric iodide, saturated, 5 drops	Slight precipitate	No change
5 per cent platinic chloride, 1 cc.	No change	Reddish yellow precipitate
5 per cent gold chloride, 1 cc.	Heavy orange precipitate	No change
Saturated silver sulphate and Ba(OH) <sub>2</sub> until alkaline	Precipitate white and brown	Black precipitate



The results of the physiological and diazo tests are recorded below.

REAGENT	PRECIPITATE		FILTRATE	
	Diazo test	Physiological	Diazo	Physiological
Picrolonic acid.....	Negative	Negative	Positive	Positive
Cadmium chloride.....	Negative	Negative	Positive	Positive
I and KI.....	Negative	Negative	Positive	Negative
Platinic chloride.....	Negative	Negative	Positive	Positive (?)
Gold chloride.....	Negative	Negative	Positive	Positive
Ag <sub>2</sub> SO <sub>4</sub> and Ba(OH) <sub>2</sub> .....	Positive	Positive	Positive	Positive

The results again confirm the former findings as to picrolonic acid and also again suggest a similarity to imidazol derivatives in that the precipitation by silver in an alkaline solution is positive and in that the diazo reaction usually parallels the activity.

This precipitation by silver sulphate and barium hydrate was repeated later on larger quantities of material. It was found that not only was the activity left to a large extent in the filtrate but that there seems to be a loss of activity by this treatment.

#### STUDIES WITH IMMISCIBLE SOLVENTS

An active solution obtained by the mercuric chloride precipitation process was rendered alkaline to litmus by sodium hydroxide and then shaken with various immiscible solvents. These solvents were later shaken with dilute aqueous hydrochloric acid and the latter concentrated under diminished pressure, diluted to definite volume and tested physiologically. In every case a precipitate formed in the alkaline aqueous solution; this was filtered off and found physiologically inactive. The results on the immiscible solvents show that the gastrin is not soluble in carbon disulphide, chloroform, benzene, ether and ethyl acetate. The only immiscible solvent by means of which we were able to extract the activity from an alkaline solution is amyl alcohol. The amyl alcohol extraction was slow but clear-cut. We used Kahlbaum's purest grade amyl alcohol for the first trials and found that careful controls thereon showed no activity. Later we used the same alcohol as such or after recovery by shaking with alkaline solutions, removing the water by anhydrous potassium carbonate and fractionating by distillation. The discovery of the solubility in amyl alcohol was next applied on pure histamine solutions and there also we found that the free base can be removed from alkaline aqueous solutions by means of

this solvent.<sup>1</sup> This was shown by means of the diazo-benzene sulphonic acid reaction. An attempt was made to carry on a continuous Soxhlet extraction of a dry alkaline mixture of crude gastrin, but the amyl alcohol extract became very much discolored, gave indications of resin formation, and contained very little or no activity. Very likely the activity was lost in the prolonged extraction at the boiling temperature of amyl alcohol.

The similar solubilities of gastrin and histamine in amyl alcohol are suggestive, and indicate that the substances are of similar character. They do not appear to be identical because, as shown above, the gastrin solution obtained from an amyl alcohol extract does not yield a physiologically active precipitate with picrolonic acid as does histamine.

#### STABILITY STUDIES

*Action of alkaline solutions.* To 10 cc. of the same preparation as used for the immiscible solvent studies we added 5 cc. 10 per cent sodium hydroxide in excess beyond the neutral point and then evaporated to dryness on the water bath. The material was next redissolved in water, neutralized by dilute hydrochloric acid and diluted to definite volume. This solution was compared with one treated in the same way only the evaporation was conducted with the solution in a neutral condition. The protocol below gives the physiological responses from the material and shows the destructive action of alkali on gastrin.

#### *Dog III. Pawlow accessory stomach, May 12, 1915*

TIME	JUICE	HYDROCHLORIC ACID		REMARKS
		Free	Total	
	cc.	per cent	per cent	
9:34-10:34 a.m.	2.3	0.12	0.17	Some mucin Clear
10:34-11:34 a.m.	2.0	Trace	0.09	
11:34 a.m.- 12:34 p.m.	1.0	Trace	0.05	
12:34 p.m.	Injected 1 cc. of the solution evaporated in neutral condition			
12:34-1:34 p.m.	11.0	0.41	0.45	Clear
1:34-2:34 p.m.	1.2	0.36	0.39	Clear
2:34 p.m.	Injected an equivalent amount evaporated in alkaline solutions*			
2:34-3:34 p.m.	5.7	0.35	0.36	Clear
3:34-4:34 p.m.	0.6	0.24	0.27	Clear

\* Slight loss in injection, not enough to account for the difference in secretion.

<sup>1</sup> This property was later applied in the quantitative separation of histamine and histidine as recently published by Koessler and Hanke. *Journ. Biol. Chem.*, xxxix, 521.

*Action of acids.* To a solution of gastrin obtained from the decomposition of mercuric chloride precipitate, there was added an equal volume of concentrated hydrochloric acid (sp. gr. 1.19) and this was boiled for twenty-four hours under a reflux condenser protected from oxygen by a pyrogallol seal. The acid was removed by evaporating to dryness under diminished pressure, redissolving the residue in water and again evaporating in the same way. The solution finally was diluted to the original volume. In the protocol below Sf is the original solution and SfA the acid treated material.

*Dog III. Pawlow accessory stomach, March 25, 1915*

TIME	JUICE	HYDROCHLORIC ACID		REMARKS
		Free	Total	
	cc.	per cent	per cent	
9:18-10:18 a.m.	1.9	Negative	Bare trace	Mucus
10:22 a.m.	Injected 1 cc. Sf			
10:22-11:22 a.m.	7.4	0.29	0.32	
11:22-11:52 a.m.	0.8	0.27	0.33	
11:52 a.m.	Injected 1 cc. SfA $\approx$ 1 cc. Sf			
11:52 a.m.-				
12:52 p.m.	9.2	0.36	0.40	
12:52-1:22 p.m.	1.5	0.35	0.36	

In another experiment a similar preparation was boiled for forty-nine hours, without protection from oxygen, with 20 per cent hydrochloric acid. The physiological results confirm the findings given above. This remarkable stability in acid solution is further confirmed by our experience with gastrin solutions kept for months in a distinctly acid solution under a layer of toluol.

COLOR REACTIONS OF GASTRIN SOLUTIONS

It is to be noted that in the precipitation studies reported above one finds the diazo benzene sulphonic acid reaction positive in very nearly every case in which we found activity. This was found to be generally true in all our separations and, in fact, this color reaction was frequently used as a rapid test for detecting complete washing of precipitates to be discarded. The intensity of this color reaction always was found to parallel intensity of the physiological activity. It again suggests a similarity in the chemical character of histamine and gastrin. Other color reactions were carried out on variously purified gastrin

solutions with the conclusions given below. Negative reactions were obtained by ninhydrin, di-methyl amido benzaldehyde, glyoxylic acid and furfural condensation. The Millon reaction was very faintly positive with a heavy precipitate. Knoop's bromine reaction was positive as with histidine, and the biuret test was positive but developed slowly on a solution which had not been hydrolyzed with hydrochloric acid. After hydrolyzing for 49 hours the activity remained, but the biuret reaction now was negative while the bromine and diazo reactions remained positive. The xantho-proteic acid reaction was very faintly positive on the mercury precipitated gastrin preparation.

#### PHYSIOLOGICAL STUDIES WITH SUBSTANCES OF KNOWN COMPOSITION

It was thought that by following the secretion of gastric juice after the injection of various substances of possible biological importance, but of known composition, we might be able to throw some light on the probable chemical character of the active substance in the gastric mucous membrane. The results of these studies are given in the table below.

TABLE 1

SUBSTANCE	TOTAL DOSE	NUMBER OF EXPERIMENTS	PHYSIOLOGICAL RESULTS		
			Posi- tive	Nega- tive	Doubt- ful
	mgm.				
Triethylamine HCl.....	10.0	3		2	1
Tetraethylamine HCl.....	10.0	1		1	
Cystin.....	20.0	4	1	2	1
Tryptophane.....	20.0	4		4	
Creatine.....	7.5	4		4	
Creatinine.....	7.5	5		5	
Choline chloride.....	10.0-20.0	16	5	10	1
Histidine.....	10.0	2		2	
Methylimidazol HCl.....	10.0	1		1	
Hydroxymethylimidazol HCl.....	10.0	1		1	
Guanidine HCl.....	10.0	5		4	1
Glucosamine HCl.....	20.0	4		4	
Histamine.....	0.25-1.0	Numerous	All	0	0

Apparently the only substances which have caused a secretion are choline and histamine. However the response from choline is so uncertain and so slight as compared with gastrin solutions and other tissue extracts that the physiological action of these extracts cannot be attrib-

uted to the presence of choline as was at one time claimed by various investigators (3). It is of interest to note that we never were able to cause a secretion of gastric juice by feeding gastrin by mouth in two- to threefold doses, nor have we ever been able to detect gastrin activity in dog or human gastric juice even after concentrating under diminished pressure and injecting intramuscularly.

#### GENERAL DISCUSSION OF THE RESULTS

Our studies thus far indicate that the active substance in gastrin solutions obtained from hog gastric mucous membrane is a very stable substance in acid solutions, that it is not easily oxidized in acid or neutral solutions, but that it is destroyed appreciably by heating with strong bases in water solution. The active substance does not lose its activity by prolonged acid hydrolysis and thus appears to be a distinctly basic substance as shown by its precipitation from acid solution by mercuric chloride and by its behavior toward immiscible solvents in the alkaline and acid conditions. It is not precipitated in the purine base precipitation method. The constant diazo reaction also suggests a basic substance and is particularly interesting in that pilocarpine is also an imidazol derivative. All of these properties suggest a similarity to pancreatic secretin as reported by others, with the exception that pancreatic secretin is generally considered to be more labile, especially in an atmosphere of oxygen (4). The properties enumerated above, as well as the physiological action, further suggest the identity of gastrin and histamine, but thus far we have not been able to precipitate the activity of a gastrin solution by means of picric or picrolonic acid. The work of Abel and Kubota (5) also suggests that histamine is the active constituent in our extracts, but as their work was based mostly on physiological evidence the proof is by no means conclusive. Koessler and Hanke<sup>2</sup> have in fact not been able to confirm the work of Abel and Kubota in that they did not find histamine by careful chemical search in casein nor in the fresh ox hypophysis. The results of Abel and Kubota (5), Koessler and Hanke (6), Tomaszewski (7), Fawcett, Hackett and Rahe (8), as well as our own results indicate the very wide distribution of substances, probably of basic character and similar in physiological action, in many tissues and possibly in the proteins themselves. It is very interesting indeed that we find these substances in relatively high concentrations in the same organs in which ammonia is generally most

<sup>2</sup> Unpublished verbal communication.

prominent immediately after death. It may also be recalled that these substances do not act physiologically when given by mouth and that they appear to be absent from gastric juice. The substance or substances in question may later be found to be identical or very closely related, possibly peptamides of very closely related structures.

#### CONCLUSIONS

1. Our results suggest a striking similarity between histamine and gastrin.
2. Gastrin appears to be more stable than secretin is reported to be, although they are similar in many respects.
3. Gastrin has not been precipitated by picric or picrolonic acid.
4. Gastrin appears to be a basic imidazol derivative which can be extracted as the free base by amyl alcohol.
5. Thus far histamine and pilocarpine are the only known imidazol derivatives which stimulate the gastric mechanism to secretion.
6. Choline acts with uncertainty and then only faintly as compared with histamine or gastrin.

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## ESSENTIALS IN MEASURING EPINEPHRIN OUTPUT WITH FURTHER OBSERVATIONS ON ITS RELATION TO THE RATE OF THE DENERVATED HEART

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The measurement of the rate at which epinephrin is given off from the adrenals under any given conditions necessarily involves the measurement of a mass and a time, since that rate is the quantity of epinephrin liberated per unit of time. It seems absurd to set this down in so many words. And the absurdity would strike every reader if our subject were the rate of output of blood from the left ventricle, the rate of output of carbon dioxide from muscles, the rate of hydrolysis of starch by amylase or the rate of excretion of urea by the kidney. But curiously enough, in the case of epinephrin some writers seem to assume that the mathematical conditions which govern the measurement of every velocity can be circumvented. And quantitative conclusions as to the influence of this or that factor upon the rate of epinephrin output have been confidently deduced from reactions which are not even specific qualitative reactions for epinephrin (such as the paradoxical dilatation of the pupil or acceleration of the denervated heart) and from observations which did not comprise a single measurement of the quantity of epinephrin given off per unit of time. Indirect qualitative methods may have value in corroborating the results of direct quantitative methods, but in case of conflict the presumption must be in favor of the latter. We have recently (1) had occasion to call attention to the bearing of this generally accepted principle upon certain discrepancies in the literature in regard to the influence of asphyxia upon the rate of epinephrin output.

The straightforward way of measuring the mass of epinephrin given off per unit of time is to collect blood from the adrenal veins for a known time and to assay its concentration in epinephrin. Various methods of obtaining the blood may be adopted depending on the kind of animal

and other conditions of the experiments. Thus, in good-sized dogs a cannula may be inserted into the lumbo-adrenal vein on one side extraperitoneally, and blood collected from one adrenal. In cats the most convenient method is to form a cava pocket. This is a method which has been employed by a number of investigators, including Hoskins and McClure<sup>1</sup> (2) who, in addition to the cava and the veins entering it which Biedl (3) tied off, ligated the abdominal aorta. We also prefer to tie the abdominal aorta and renal arteries, in order to prevent too much blood from stagnating in the hind end of the animal and the kidneys when the cava and renal veins have been ligated. The arteries are tied a little time before the veins, so that as much blood as possible may drain out of the occluded parts. There is no obvious reason why the exclusion of the hind legs and kidneys should affect the output or the nervous mechanism governing it. Indeed, the better blood pressure maintained after ligation of these vessels ought to favor rather than to hinder any reflex nervous effects elicited, e.g., by stimulation of the central end of sensory nerves or any excitatory effects upon nerve centers exerted by such conditions as asphyxia, since the conductivity of the reflex arcs and the excitability of the nerve centers must be better maintained when the blood flow is increased. The ordinary spinal reflexes are well obtained in the parts whose circulation has not been interfered with, and indeed in the hind end for some time after ligation of the vessels (see, e.g., protocol of cat 436).

When Doctor Cannon (4) attributes to the peculiar method adopted by us for collecting the blood our failure to corroborate his results, he does not explain how our method is unfavorable to the eliciting of the reaction. He attempts to show that the output of epinephrin measured by us in adrenal vein blood, collected from the cava and assayed on rabbit intestine (and uterus) segments, is an artificial phenomenon. He says "the effect of opening the abdominal cavity, clamping off the inferior cava and repeatedly manipulating the abdominal contents,

<sup>1</sup> The epinephrin output determined by these observers in 5 dogs by assay of adrenal blood, collected in the manner described, on rabbit intestine segments agrees well with our results. If the 1:1,000,000 adrenalin solution, in terms of which the output is expressed, is assumed to contain about 75 per cent of base (it was probably not assayed) the average output would be 0.00019 mgm. per kgm. per minute. Our average for 17 dogs was 0.00022 mgm. per kgm. per minute. The average weight of our dogs was exactly half that of the dogs employed by Hoskins and McClure, and there is reason to believe that in the larger dogs the output of epinephrin per kilogram of body weight is somewhat less than in the smaller.



either in pressing blood out of the inferior cava or withdrawing it by syringe, must be examined." We do not press blood out of the inferior cava nor do we withdraw it by syringe, if that is a more serious insult to the animal, nor do we repeatedly manipulate the abdominal contents. We open the abdominal cavity, prepare the cava pocket and clamp it off when blood is to be collected from the cannula inserted in the lower end of the pocket. We have examined the influence of several of the factors mentioned upon the output and have not found any effect detectable by our methods (5), (6).

*The alleged influence of opening the abdomen upon epinephrin secretion.* Doctor Cannon does not state the result of his examination. All he says is that "fully twenty years ago Bayliss and Starling called attention to the profound effect which opening the abdominal cavity has on the intestines in causing them to become absolutely motionless. These well-established facts make an interesting commentary on the use of the cava pocket as a mode of obtaining evidence of normal or natural secretion." He proceeds, "There is no doubt that secretion from the adrenal medulla is subject to impulses delivered by the splanchnic nerves and there is no doubt that opening the abdominal cavity under anesthesia results in a discharge of impulses along these nerves. The adrenal glands, therefore, are continuously and abnormally stimulated if the abdomen is opened." If Bayliss and Starling had found that opening the abdomen caused the setting up of inhibitory impulses for the intestines, this would, of course, form a complication in the study of the intestinal movements under these conditions. Despite this, Cannon accepts their results as, so far as we are aware, everybody else does. But because he imagines that opening the abdomen sets up inhibitory impulses for the intestinal movements which, however, did not prevent Bayliss and Starling from making trustworthy observations, he concludes that our results on a totally different object, the adrenals, are completely vitiated by impulses set up in an altogether different group of nerve fibers, the epinephrin-secreting fibers, although neither he nor anybody else has ever shown that opening the abdomen influences them in the least. The only commentary we need make upon this is that Bayliss and Starling (7) do not call attention at all to the effect of opening the abdomen in causing the intestines to become absolutely motionless. On the contrary, they state that when they opened the abdomen in the warm-saline bath they found the intestines collapsed and absolutely motionless if the splanchnic nerves were intact. They drew the conclusion, not that

the opening of the abdomen caused the inhibitory impulses to the intestines to be set up but that these impulses were already descending the splanchnics before the abdomen was opened. Singularly enough, Cannon quotes their statement *verbatim*: "These facts suggest that in the *intact* animal, at any rate under the conditions of our experiment, tonic or reflex influences are continuously descending the splanchnic nerves, inhibiting the activity of the intestines." To be logical, then, he ought to conclude that since the impulses which pass to the intestines along the splanchnics are *not* called into existence by opening of the abdomen but are already present, impulses descending the splanchnics to the adrenals and sustaining a normal epinephrin output must also be present in the intact animal. If opening the abdomen so completely deranges the action of abdominal viscera, including the adrenals, a very large part of our supposed physiological knowledge must be wiped out. The truth is, of course, that we must neither assume in any particular case that opening of the abdomen is indifferent to the experiment nor that it absolutely contraindicates it, but must always test, as far as possible, whether and in what direction this operation, as well as the other experimental conditions, influence the result. We have pointed out elsewhere (22) that the relatively narrow range within which the output, (as measured by observers, including ourselves, who have employed methods correct in principle), varies with different anesthetics and different operations suggests strongly that what we term the normal or ordinary spontaneous output is not initiated or sustained by the trauma or the anesthesia.

To emphasize his criticism of our method of obtaining adrenal vein blood by opening the abdomen, Cannon states that the acceleration of the denervated heart, caused by stimulation of the central end of the sciatic, is hardly ever observed after opening the abdomen, although asphyxia still gives the reaction. He apparently considers this remark so important that he italicises the following sentence, "in the entire series of cases with opened abdomen there was only one in which sensory stimulation caused any effect ascribable to adrenal secretion." Since we have shown (8) that this heart reaction has no significance as an indication of augmented epinephrin output, the statement that acceleration on sciatic stimulation is rarely obtained after the abdomen has been opened would, even if true, have no bearing upon the question at issue. It is easy to demonstrate, however, that the reaction is readily obtained not only soon after the abdomen has been opened but long thereafter, and when the abdominal aorta, renal vessels and cava

have been tied or clipped off in making the cava pocket. Indeed, there was some indication that the reaction might be greater after clipping the abdominal aorta, presumably owing to the better blood flow in the nervous centers (see, e.g., protocol of cat 437 in the previous paper (8). There is plenty of evidence in the protocols of that paper that opening of the abdomen does not interfere with the reaction in question, and this is further illustrated here in figures 1 to 4.

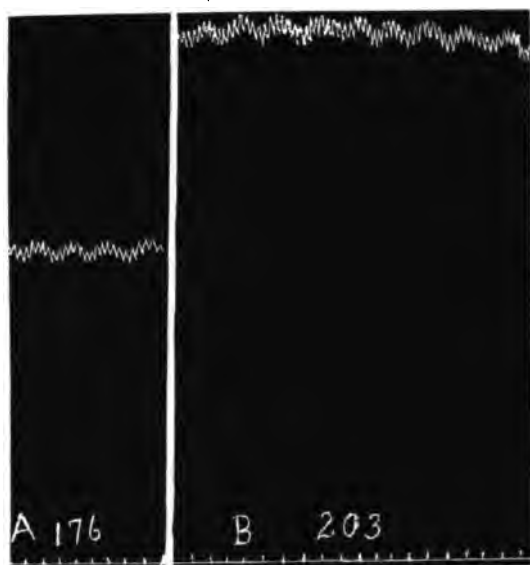


Fig. 1. Parts of blood pressure tracing from cat 175. *A*, before and *B*, a portion commencing 24 seconds after beginning sciatic stimulation; (abdomen opened, left splanchnic cut nearly 2 hours). In all figures line of zero pressure corresponds with time trace; time in seconds; numbers above time trace represent heart rate per minute.

The protocols of the experiments from which these tracings are taken have already been published (8). In figure 1 are reproduced two portions of the blood pressure tracing (from cat 175), one just before, *A*, and the other during stimulation of the sciatic, *B*. The pulse rate was increased by nearly 30 beats per minute. The abdomen had not only been opened almost 2 hours before, and the left splanchnic nerve cut, but the intestines had been purposely manipulated. The heart reaction had been obtained many times after opening the abdomen,

both by excitation of the sciatic and by excitation of the left splanchnic before this tracing was taken, but an excellent rise of pressure was still caused when either of these nerves was stimulated. Stimulation of the sciatic before the abdomen was opened and with both splanchnics intact gave an acceleration of 22 beats in one observation and 25 beats in another.

Figure 2 from another cat (177) shows an acceleration of almost 40 beats produced by sciatic stimulation after opening the abdomen and

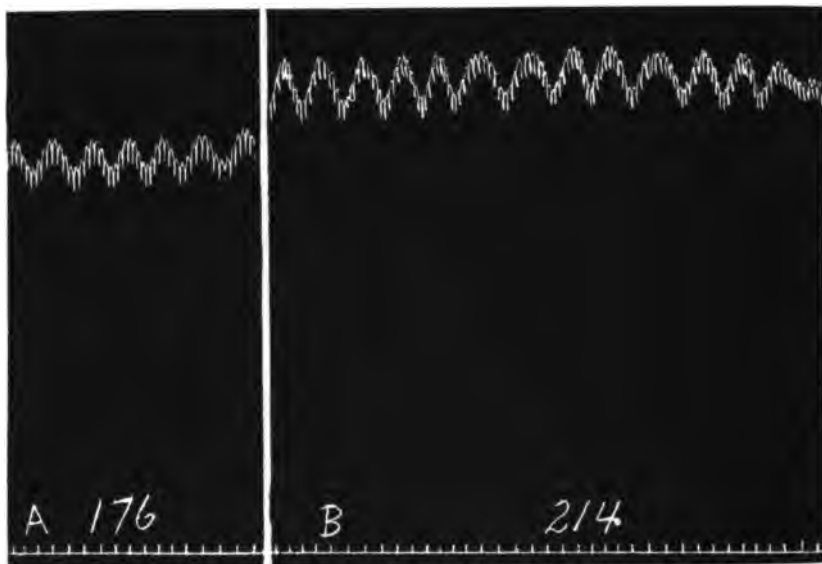


Fig. 2. Parts of blood pressure tracing from cat 177. A, before and B, a portion commencing 32 seconds after beginning of sciatic stimulation (abdomen open). Reduced one-nineteenth.

section of one splanchnic. Before the abdomen was opened, stimulation of the sciatic caused a maximum acceleration of 43 beats, with both splanchnics intact.

In figure 3 are reproduced portions of a curve (from cat 179) demonstrating a maximum acceleration of 34 beats, produced by sciatic stimulation before opening of the abdomen. One splanchnic had been previously cut extraperitoneally. After the abdomen was opened, a number of sciatic and splanchnic stimulations having been made in the meantime, stimulation of the sciatic caused an acceleration of 24

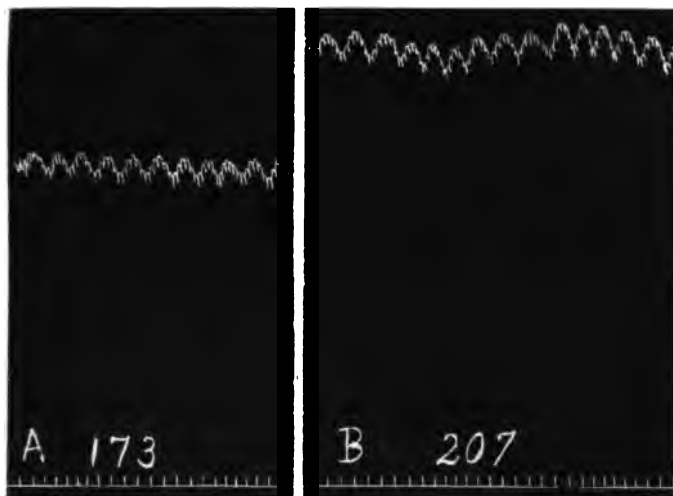


Fig. 3. Parts of blood pressure tracing from cat 179. *A*, before and *B*, a portion commencing 32 seconds after beginning of sciatic stimulation (left splanchnic cut).

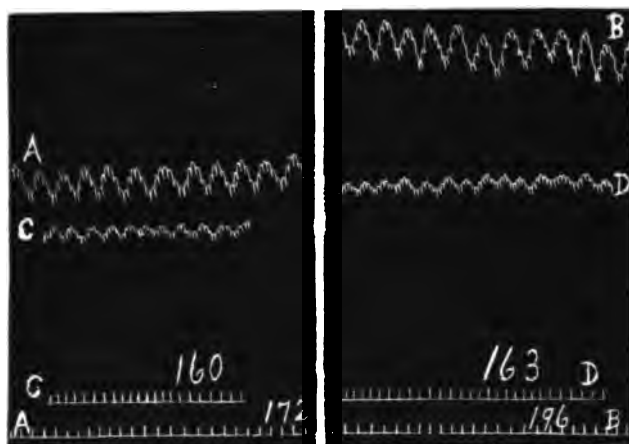


Fig. 4. Parts of blood pressure tracing from cat 179. *A*, before and *B*, a portion commencing 25 seconds after beginning of sciatic stimulation (left splanchnic cut and abdomen opened). *C*, before and *D*, a portion commencing 23 seconds after beginning of sciatic stimulation (both splanchnics cut).

beats (fig. 4, *A* and *B*). In this, as in all the other experiments, so long as sciatic stimulation gave a good rise of blood pressure, it gave a substantial acceleration of the heart. Later on in the experiment the second splanchnic was cut. The blood pressure fell to 44 mm. of mercury, and stimulation of the sciatic now caused little or no acceleration of the heart and only an insignificant rise of blood pressure (fig. 4, *C* and *D*).

If Cannon believes that the presence of the reaction indicates increased epinephrin output, it is incumbent upon him to explain why in adrenal blood collected at a time and under conditions when this reaction is well obtained, we are unable to detect, by a direct and sensitive method of assay (intestine segments), any sensible increase in the rate



Fig. 5. Parts of blood pressure tracing from cat 436. *A*, before asphyxia for 60 seconds; *B*, 7 seconds before end and 16 seconds after end of asphyxia; *C*, a portion commencing 40 seconds after end of asphyxia (abdominal aorta and renal vessels tied). Reduced to four-fifths.

of output. In the case of asphyxia it is still more necessary that he should explain why we cannot detect such great increases in the epinephrin output as he assumes to occur. For he admits that the acceleration of the denervated heart associated with asphyxia is not abolished by opening the abdomen. We can confirm this entirely, and can add that the reaction can also be obtained after ligation of the abdominal aorta, renal arteries and veins and other vessels, as done in the formation of a cava pocket for the collection of adrenal vein blood. This is illustrated in figure 5 (from cat 436).

*Condensed protocol.* Cat 436; male; weight 2.26 kgm.

Left superior cervical ganglion excised 28 days previously. Under urethane (6 grams) cut vago-sympathetics, excised stellate ganglia, prepared central end of left sciatic for stimulation. The anesthesia was very deep and considerable depression of respiration and circulation was present from the start.

		<i>Rate</i>	<i>Pressure</i>
12:42 p.m.	Before sciatic stimulation.....	203	56
	6 seconds after beginning stimulation.....	206	62
	20 seconds after beginning stimulation.....	210	64
	40 seconds after beginning stimulation.....	204	64
12:52 p.m.	Before asphyxia for 45 seconds.....	135	26
	Just after beginning asphyxia.....	140	30
	10 seconds after beginning asphyxia.....	162	46
	26 seconds after beginning asphyxia.....	195	45
1:05 p.m.	Opened abdomen, tied abdominal aorta, renal arteries and veins		
1:10 p.m.	Before asphyxia for 45 seconds.....	184	49
	Just after beginning asphyxia.....	180	54
	15 seconds after beginning asphyxia.....	181	62
	Just after end of asphyxia.....	198	60
1:15 p.m.	Intravenous injection of 50 cc. Ringer's solution		
	Before Ringer injection.....	200	53
	After Ringer injection.....	204	80
1:35 p.m.	Before asphyxia for 60 seconds.....	194	66
	Just after beginning asphyxia.....	199	78
	30 seconds after beginning asphyxia.....	178	56
	Just after end of asphyxia.....	212	62
	20 seconds after end of asphyxia.....	219	53
	40 seconds after end of asphyxia.....	223	54
1:40 p.m.	Tied coeliac axis and superior mesenteric artery, clipped cava; hind leg and tail reflexes still present		
1:42 p.m.	Before asphyxia for 60 seconds.....	205	63
	During first 30 seconds of asphyxia.....	201	64
	Just after end of asphyxia.....	204	65
	15 seconds after end of asphyxia.....	218	60
	30 seconds after end of asphyxia.....	220	59
1:48 p.m.	Completed cava pocket		
1:49 p.m.	Before closure of pocket.....	208	56
	During closure of pocket.....	190	42
	Just after release of pocket blood.....	189	44
	15 seconds after release of pocket blood.....	215	58

In any case how does Cannon conceive asphyxia to act upon the epinephrin output? He says a short asphyxia acts through the nervous system, a longer period of asphyxia has a direct action. Now, why should the stimulating action of asphyxia on the part of the central nervous system which presides over the adrenal epinephrin secretion be abolished by tying the abdominal aorta? The effect of its ligation is to raise the blood pressure and therefore to keep up a more efficient circulation in the central nervous system and adrenals. How does this prevent the stimulation of the nerve centers by asphyxia? It does

not do so, and the reason we do not find an increased output of epinephrin with asphyxia must be that when tested by a quantitative method, the alleged increase is non-existent or too small to be detected.

*The relation of the epinephrin output of the adrenals to the acceleration of the denervated heart associated with asphyxia.* Cannon states that tying the lumbo-adrenal veins on both sides of the adrenals may not diminish in the least the acceleration of the heart produced by asphyxia of a given duration. This seems very good evidence that the reaction cannot be a quantitative reaction for epinephrin output. It is fully in accord with this conclusion that Cannon himself sometimes obtained a very large acceleration by asphyxia after section of both splanchnics. He endeavors to explain this by assuming that prolonged asphyxia stimulates the adrenal medullary cells directly. He brings forward no real evidence that this occurs. v. Anrep (9) states that after section of both splanchnics (in the dog) no reaction is elicited by asphyxia on the blood vessels of the denervated limb, and concludes that asphyxia has no direct action upon the adrenal medulla. Pearlman and Vincent (10), however, have not been able to obtain on the denervated limb any reaction in asphyxia which could be attributed to epinephrin. If asphyxia lasting for 90 seconds can stimulate the adrenal medulla directly so as to cause enough epinephrin to be given off to accelerate the heart by 68 beats a minute after section of the splanchnics, our failure to obtain evidence of this effect in adrenal vein blood directly assayed would be more puzzling than ever. For Cannon himself admits that the asphyxial acceleration with intact splanchnics is not interfered with by opening the abdomen, and how then should opening the abdomen abolish an action of asphyxia exerted directly upon the adrenal cells? Cannon's conclusion, that asphyxia stimulates directly the adrenal medulla, is simply a misinterpretation of his result that sometimes after division of both splanchnics asphyxia may cause a quickening of the denervated heart. Even if the heart reaction were specific for epinephrin, this would only indicate that some portion of the nerve supply of the adrenals might have been spared, and would not of itself prove a direct action. But as soon as it is shown that the reaction is not specific for epinephrin, and can be obtained after removal of the adrenals, the whole argument is seen to be baseless.

Cannon cites Czubalski (11) as having "adduced evidence that asphyxia if sufficiently prolonged may have a direct stimulating action on the adrenal medulla." The paper referred to is a preliminary note in which it is stated that even after section of the cord and bulb and also after division of the splanchnics



and vagi, a rise of blood pressure can be produced beginning usually at the end of the third or beginning of the fourth minute of asphyxia. The rise of blood pressure was very often accompanied by marked slowing of the heart. Czubalski attributes the increase of pressure to adrenalin liberated by the direct action of asphyxia on the adrenal medulla. The heart, of course, was isolated as regards its extrinsic innervation, and, according to Cannon, the characteristic response of the heart thus isolated to increased epinephrin output is an acceleration. It is curious that he should find confirmation of his result on the direct action of asphyxia on the adrenals in an effect upon the heart precisely the opposite of that which he associates with epinephrin. Czubalski's assertion that the general blood of a dog after asphyxia contains so much epinephrin that its presence is demonstrated by marked inhibition of a rabbit's intestine segment, when the defibrinated blood is made to displace "a nutritive solution," is of itself sufficient to show that his conclusions are not of any value. His statement that after removal of the adrenals asphyxia no longer causes a rise of blood pressure, is invalidated by the observations of Gley and Quinquaud (13).

In one of Cannon's experiments he obtained with an asphyxia lasting 90 seconds, which is what he considers a prolonged asphyxia, an acceleration of 28 beats before section of the splanchnics. After section of the splanchnics no acceleration was produced by an asphyxia of 60 seconds, but he does not state what acceleration, if any, was caused by an asphyxial period of the same duration as the control before splanchnotomy, so that there is no way of judging whether the section of the splanchnics in this case had anything to do with the negative result. If the asphyxial rise of pressure is a factor in the acceleration, it is easy to see that section of the splanchnics or injury to them in removal or ligation of the adrenals must interfere with the reaction, apart altogether from the interference with the epinephrin output. In the figure given by Cannon (fig. 2) there seems to have been no rise of pressure after removal of the adrenals till 45 seconds from the beginning of asphyxia, whereas before removal of the adrenals the curve shows a substantial rise of pressure before this, how much earlier it is impossible to say from the section of the curve reproduced.

It is unnecessary, however, to go into details of this kind. The best proof that Cannon's supposed demonstration of the augmenting effect of asphyxia upon the epinephrin output by means of the heart reaction is fallacious is that asphyxia can elicit marked acceleration in the absence of the adrenals.

*Experiments showing that asphyxia can cause acceleration of the denervated heart in the absence of epinephrin from the adrenals.* Several instances have been given in the previous paper (8). Thus in cat 438 the heart was beating at the rate of 175 a minute. The second adrenal had been removed 35 minutes previously by the abdominal route. Asphyxia was induced for 60 seconds and the heart rate rose to 225 beats a minute, the maximum acceleration being attained after resumption of respiration. The blood pressure was 64 mm. of mercury and

did not change much. Sciatic stimulation at this time caused little if any acceleration of the heart, although earlier in the experiment and after removal of the second adrenal it gave a good acceleration. In cat 440 after removal of the adrenals and exposure of the cervical cord, which was associated with a fall of blood pressure of 20 to 30 mm. of mercury, asphyxia for 45 seconds caused an acceleration of 13 beats during the asphyxial period and of 18 beats counting from the end of asphyxia, the blood pressure rising only slightly (from 84 to 88 mm. of mercury). Stimulation of the sciatic, which had caused good accelerations (as much as 42 beats a minute) after excision of the second adrenal, had at this time only a very slight effect upon the heart rate. With a subsequent asphyxia of 30 seconds, the heart rate diminished from 216 a minute before to 203 during the asphyxia, but recovered to the initial rate on stopping the asphyxia.

In a number of experiments the preliminary dissection for excluding the adrenals from the circulation was made by the abdominal route at the beginning of the experiment, and ligatures were placed but not tied. The abdomen was then closed. After one or more periods of asphyxia with the adrenals discharging, the ligatures were tied so as to occlude the adrenal arteries, the adrenal veins and the lumbar veins just before they cross the glands. The abdomen was closed and the effect of asphyxia on the heart rate was again observed. Finally the adrenals were excised by cutting between them and the ligatures. There was no bleeding and no further ligatures were required, showing that the glands had been completely excluded from the circulation. The protocol of cat 445 is given as an example.

*Protocol.* Cat 455; female; weight, 2.27 kgm. Under ether cut vago-sympathetics, excised stellate ganglia, opened abdomen and placed ligatures in position to occlude adrenal vessels but did not tie them.

		Rate	Pressure
12:52 p.m.	Before asphyxia (30 seconds).....	284	130
	Just after end of asphyxia.....	313	118
	15 seconds after end of asphyxia.....	318	127
	30 seconds after end of asphyxia.....	304	126
12:55 p.m.	Before asphyxia (45 seconds).....	268	125
	During first 15 seconds asphyxia.....	274	130
	During next 15 seconds asphyxia.....	278	114
	During next 15 seconds asphyxia.....	295	100
	Just after end of asphyxia.....	312	120
	20 seconds after end of asphyxia.....	323	132
	35 seconds after end of asphyxia.....	316	138

	<i>Rate</i>	<i>Pressure</i>
1:05 p.m. Before asphyxia (60 seconds).....	308	102
During first 15 seconds asphyxia.....	303	105
During next 15 seconds asphyxia.....	302	96
Just after end of asphyxia.....	321	57
15 seconds after end of asphyxia.....	335	72
30 seconds after end of asphyxia.....	350	72
50 seconds after end of asphyxia.....	334	76
1:12 p.m. Tied off adrenal vessels		
1:14 p.m. Before asphyxia (40 seconds).....	283	102
During first 12 seconds asphyxia.....	287	98
Just after end of asphyxia.....	301	81
15 seconds after end of asphyxia.....	321	95
30 seconds after end of asphyxia.....	301	94
45 seconds after end of asphyxia.....	298	94
1:18 p.m. Before asphyxia (60 seconds).....	283	95
During last 15 seconds asphyxia.....	313	93
Just after end of asphyxia.....	326	96
1:23 p.m. Before asphyxia (60 seconds).....	300	105
During first 15 seconds asphyxia.....	300	109
During next 15 seconds asphyxia.....	296	98
During next 15 seconds asphyxia.....	308	98
During next 15 seconds asphyxia.....	312	98
Just after end of asphyxia.....	316	100
15 seconds after end of asphyxia.....	310	100
30 seconds after end of asphyxia.....	303	100
1:30 p.m. Excised both adrenals; prepared central end of sciatic		
1:40 p.m. Before asphyxia (35 seconds).....	287	95
During first 20 seconds asphyxia.....	285	98
Just after end of asphyxia.....	280	64
15 seconds after end of asphyxia.....	283	64
30 seconds after end of asphyxia.....	284	64
45 seconds after end of asphyxia.....	287	84
1:50 p.m. Before sciatic stimulation (6 cm.).....	264	67
Just after end of stimulation.....	286	90
15 seconds after end of stimulation.....	284	75
1:57 p.m. Before asphyxia (60 seconds).....	261	70
During asphyxia.....	264	57
Just after end of asphyxia.....	267	68
20 seconds after end of asphyxia.....	269	69
35 seconds after end of asphyxia.....	270	72
2:06 p.m. Before asphyxia (45 seconds).....	238	52
During first 15 seconds asphyxia.....	238	57
During next 15 seconds asphyxia.....	238	50
Just after end of asphyxia.....	249	48
15 seconds after end of asphyxia.....	247	44
30 seconds after end of asphyxia.....	248	44
50 seconds after end of asphyxia.....	247	60

		Rate	Pressure
2:14 p.m.	Before sciatic stimulation.....	238	49
	During first 15 seconds stimulation.....	239	63
	During next 10 seconds stimulation.....	242	64
	During next 10 seconds stimulation.....	246	65
	Just after end of stimulation.....	242	64
2:32 p.m.	Before asphyxia (45 seconds).....	244	62
	During first 25 seconds asphyxia.....	245	54
	Just after end of asphyxia.....	242	45
	20 seconds after end of asphyxia.....	240	49

It will be seen that ligation of the adrenals did not interfere with the acceleration of the denervated heart induced by asphyxia. The first asphyxia after ligation of the glands gave an acceleration of 38 beats, the second an acceleration of 43 beats per minute. The third asphyxia gave an acceleration of 16 beats, but the initial rate was already 300, which was 17 beats greater than before either of the two previous asphyxial periods. After actual removal of the adrenals in this experiment the maximum acceleration caused by asphyxia was slight (only 9 beats per minute). This, however, can have nothing to do with loss of epinephrin output since the glands had been entirely excluded from the circulation by the ligation.

We have had abundant evidence, if evidence were needed, that repeated asphyxiation is not an indifferent procedure for the heart. A heart which at first has responded by an acceleration during the asphyxial period, and usually a still greater acceleration immediately thereafter, may later on respond by some diminution in the pulse rate, becoming more manifest toward the end of the asphyxia, and succeeded by an increase on stopping the asphyxia, which may carry the rate beyond the initial value or not. In figure 2 of Cannon's paper (4) the heart rate toward the end of asphyxia (after the adrenals were tied off) was slower by 6 or 7 beats a minute than before the asphyxia. It then increased to the initial rate after respiration was resumed. As only a small portion of the curve is reproduced, it does not show whether later on the rate went beyond the initial value or not. When successive periods of asphyxia are superimposed upon a steadily declining blood pressure in the course of an experiment the loss or diminution of the heart reaction is apt to be particularly evident.

A good acceleration was still given when the sciatic was stimulated after excision of the adrenals, accompanied by a fair rise of pressure. It is scarcely necessary to point out that it is impossible to reconcile these results with Cannon's conclusion that the acceleration of the denervated heart is an index of the increased output of epinephrin from the adrenals caused by stimulation of the sciatic and by asphyxia. In cat 455 the heart rate after section of the vago-sympathetics and excision of the stellate ganglia was exceptionally great.

In the next experiment to be cited the heart rate was unusually low, not much more than half the rate in cat 455 and the blood pressure was also only about half.

*Protocol.* Cat 453; male; weight, 2.51 kgm. Under urethane (5 grams) cut vago-sympathetics, excised stellate ganglia, opened abdomen and placed ligatures in position to occlude adrenal vessels but did not tie them.

		Rate	Pressure
11:35 a.m.	Before asphyxia (45 seconds).....	150	66
	During first 20 seconds asphyxia.....	152	66
	During next 20 seconds asphyxia.....	152	72
	Just after end of asphyxia.....	153	76
	20 seconds after end of asphyxia.....	155	68
	35 seconds after end of asphyxia.....	156	71
11:38 a.m.	Before asphyxia (60 seconds).....	153	63
	During first 15 seconds asphyxia.....	151	63
	During next 20 seconds asphyxia.....	151	69
	During next 15 seconds asphyxia.....	157	85
	Just after end of asphyxia.....	166	77
	25 seconds after end of asphyxia.....	166	80
11:45 a.m.	Tied off adrenal vessels		
11:46 a.m.	Before asphyxia (60 seconds).....	143	57
	During first 20 seconds asphyxia.....	144	60
	During next 30 seconds asphyxia.....	147	82
	Just after end of asphyxia.....	151	66
	25 seconds after end of asphyxia.....	153	70
11:50 a.m.	Before asphyxia (85 seconds).....	143	59
	During first 30 seconds asphyxia.....	145	68
	During next 30 seconds asphyxia.....	146	76
	During next 20 seconds asphyxia.....	152	64
	Just after end of asphyxia.....	159	76
	25 seconds after end of asphyxia.....	160	74
	40 seconds after end of asphyxia.....	156	78
12:00 m.	Prepared central end of sciatic for stimulation		
12:02 p.m.	Before sciatic stimulation (5 cm.).....	153	63
	During sciatic stimulation.....	174	106
	Just after end of stimulation.....	181	75
	25 seconds after end of stimulation.....	171	75
12:10 p.m.	Before asphyxia (55 seconds).....	157	62
	During first 25 seconds asphyxia.....	158	64
	During next 30 seconds asphyxia.....	163	70
	Just after end of asphyxia.....	163	56
	20 seconds after end of asphyxia.....	170	63
12:15 p.m.	Excised both adrenals		
12:16 p.m.	Before sciatic stimulation (5 cm.).....	156	57
	During first 20 seconds stimulation.....	166	77
	During next 20 seconds stimulation.....	183	72
	Just after end of stimulation.....	171	53

		<i>Rate</i>	<i>Pressure</i>
12:22 p.m.	Before asphyxia (60 seconds) . . . . .	150	51
	During first 20 seconds asphyxia . . . . .	150	54
	During next 20 seconds asphyxia . . . . .	151	60
	During next 15 seconds asphyxia . . . . .	157	42
	Just after end of asphyxia . . . . .	153	41
	30 seconds after end of asphyxia . . . . .	155	47
12:30 p.m.	Two more observations with asphyxia gave in the first a maximum acceleration of 6 beats, in the other none; sciatic stimulation caused no acceleration; blood pressure had fallen to 48 mm., and during asphyxia to 33 mm. of mercury.		

The greatest acceleration caused by asphyxia in cat 453 before ligation of the adrenals was 13 beats a minute; after ligation it was 17 beats a minute. After excision of the glands, already completely excluded from the circulation, the greatest acceleration obtained with asphyxia was 7 beats per minute, and at the end of the experiment practically no acceleration was caused. It is obvious that the absence of the reaction in the last observation could have nothing whatever to do with the absence of the epinephrin output of the adrenals. Sciatic stimulation before excision of the adrenals, but after their exclusion from the circulation, gave an acceleration of 28 beats, and after excision of the glands an acceleration of 27 beats per minute. How is it possible to maintain that these accelerations are due to increased output of epinephrin from the adrenals, or that the failure to obtain them is due to elimination of the epinephrin output? The experiment proves clearly that the acceleration caused by asphyxia cannot be due to the same factors as the acceleration caused by sciatic stimulation, since the latter is unchanged after excision of the adrenals. In other experiments an asphyxial acceleration has been obtained at a time when little or no acceleration was elicited by stimulation of the sciatic. Figure 6 gives portions of the blood pressure curve from cat 453, showing the effect of stimulating the sciatic before and after excision of the adrenals and the effect of asphyxia after the excision.

In figures 7 and 8 are reproduced portions of the tracings from cat 456, to show the effect of asphyxia before the adrenals were tied off (maximum acceleration 22 beats per minute), after the adrenals were tied off (maximum acceleration 25 beats per minute), after the adrenals were excised (maximum acceleration 13 beats a minute), and later on (no acceleration). As shown in the protocol, ligation of the adrenals did not alter the position of the maximum acceleration any more than its absolute amount. Counts of successive portions of the curves

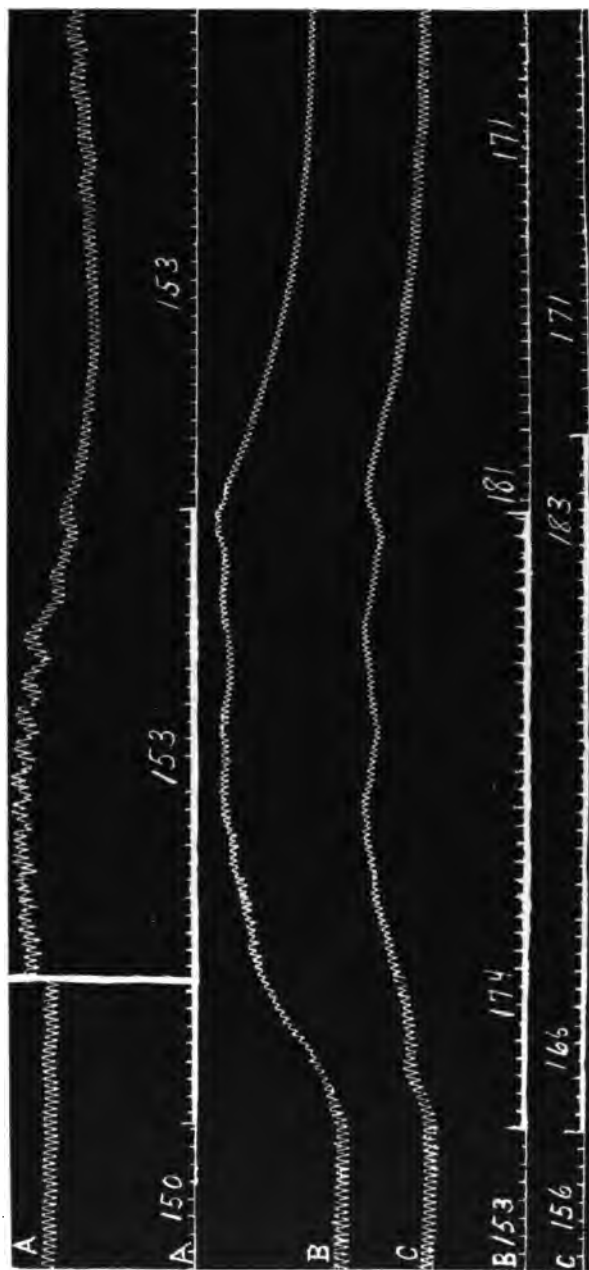


Fig. 6. Parts of blood pressure tracing from cat 453. A, asphyxia for 60 seconds, after excision of adrenals (26 seconds cut out of trace to save space); B, sciatic stimulation before, and C, after excision of adrenals. Reduced to three-fourths.

are given to establish this point, which is no more in harmony with the view that the acceleration is an index of epinephrin output than is the possibility of eliciting an undiminished acceleration after exclusion of the adrenals. Naturally, when, after ligation of the vessels, asphyxia was associated with a considerable fall of blood pressure no acceleration might be seen until respiration was resumed (Fig. 7). After re-

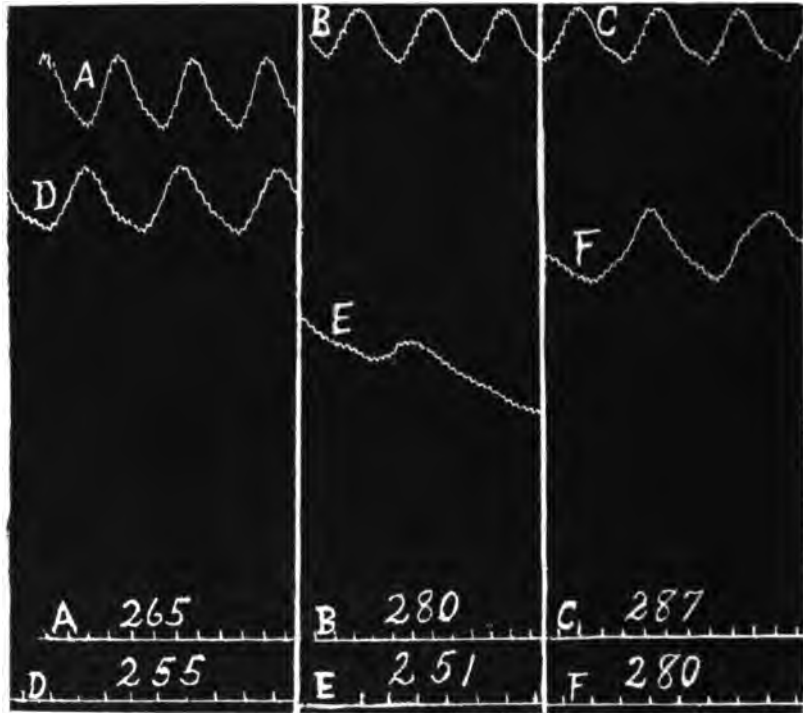


Fig. 7. Parts of blood pressure tracing from cat 456. A, before asphyxia, B, a portion just before end, and C, a portion commencing 18 seconds after end of asphyxia for 45 seconds, before tying adrenal vessels. D, before asphyxia, E, a portion just before end of asphyxia and F, a portion commencing 27 seconds after end of asphyxia for 45 seconds, after tying adrenal vessels.

peated asphyxiation the effect on the heart rate disappeared, although sciatic stimulation still caused a moderate acceleration (10 beats per minute). Again, it would be absurd to attribute the failure of the asphyxia reaction to elimination of the adrenals, since they were already



eliminated by the ligation and were cut out without bleeding, and since a fair acceleration was caused by asphyxia for some time after their removal.

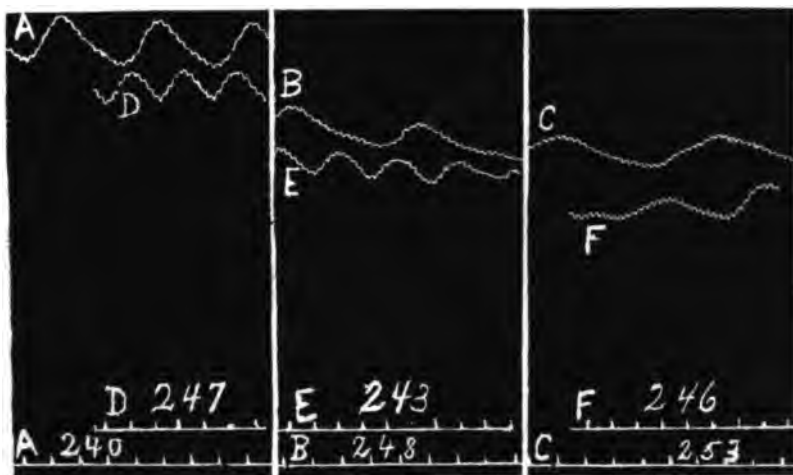


Fig. 8. Parts of blood pressure tracing from cat 456. *A*, before asphyxia, *B*, a portion just before end and *C*, a portion commencing 20 seconds after end of asphyxia for 45 seconds, after excision of adrenals. *D*, before asphyxia, *E*, a portion just before end and *F*, a portion commencing 21 seconds after end of asphyxia for 60 seconds, nearly half an hour after excision of adrenals.

*Protocol.* Cat 456; young female; weight, 1.55 kgm. Under urethane (3 grams) sectioned vago-sympathetics, excised stellate ganglia, opened abdomen and placed ligatures in position to occlude adrenal vessels but did not tie them.

		Rate	Pressure
11:53 a.m.	Before asphyxia (45 seconds).....	265	145
	During first 15 seconds asphyxia.....	270	156
	During next 30 seconds asphyxia.....	278	163
	Just after end of asphyxia.....	282	161
	15 seconds after end of asphyxia.....	286	159
	30 seconds after end of asphyxia.....	287	156
	50 seconds after end of asphyxia.....	283	150
11:58 a.m.	Tied off adrenal vessels		
12:00 m.	Before asphyxia (45 seconds).....	255	130
	During first 15 seconds asphyxia.....	260	132
	During next 15 seconds asphyxia.....	259	114
	During next 15 seconds asphyxia.....	252	80
	Just after end of asphyxia.....	269	108
	15 seconds after end of asphyxia.....	278	114
	30 seconds after end of asphyxia.....	279	126
	45 seconds after end of asphyxia.....	280	126

		<i>Rate</i>	<i>Pressure</i>
12:10 p.m.	Excised both adrenals		
12:12 p.m.	Before asphyxia (45 seconds) . . . . .	244	111
	During first 15 seconds asphyxia . . . . .	245	116
	During next 20 seconds asphyxia . . . . .	245	102
	During next 10 seconds asphyxia . . . . .	247	94
	Just after end of asphyxia . . . . .	251	94
	20 seconds after end of asphyxia . . . . .	253	98
	40 seconds after end of asphyxia . . . . .	253	98
12:15 p.m.	Before asphyxia (45 seconds) . . . . .	240	110
	During first 20 seconds asphyxia . . . . .	244	100
	During next 20 seconds asphyxia . . . . .	249	88
	Just after end of asphyxia . . . . .	248	80
	20 seconds after end of asphyxia . . . . .	253	88
	40 seconds after end of asphyxia . . . . .	252	92
12:18 p.m.	Prepared central end of sciatic for stimulation		
12:20 p.m.	Before sciatic stimulation (6 cm.) . . . . .	240	88
	During sciatic stimulation . . . . .	252	140
	Just after end of stimulation . . . . .	251	108
12:23 p.m.	Before asphyxia (through 3 ft. tube) (60 seconds) . . . . .	239	110
	During first 20 seconds asphyxia . . . . .	238	103
	During next 20 seconds asphyxia . . . . .	239	90
	During next 20 seconds asphyxia . . . . .	242	80
	Just after end of asphyxia . . . . .	241	67
	20 seconds after end of asphyxia . . . . .	244	83
	45 seconds after end of asphyxia . . . . .	247	92
12:30 p.m.	Before asphyxia (105 seconds) interrupted by 5 seconds respiration each half minute . . . . .	240	96
	During first 20 seconds asphyxia . . . . .	242	90
	During next 20 seconds asphyxia . . . . .	243	85
	During next 20 seconds asphyxia . . . . .	245	86
	During next 15 seconds asphyxia . . . . .	244	80
	During next 20 seconds asphyxia . . . . .	248	82
	Just after end of asphyxia . . . . .	246	80
12:34 p.m.	Before sciatic stimulation (5 cm.) . . . . .	242	88
	During first 15 seconds stimulation . . . . .	247	106
	During next 20 seconds stimulation . . . . .	252	120
	Just after end of stimulation . . . . .	250	102
	20 seconds after end of stimulation . . . . .	247	90
12:37 p.m.	Before asphyxia (60 seconds) . . . . .	247	89
	During first 20 seconds asphyxia . . . . .	245	76
	Just after end of asphyxia . . . . .	243	57
	20 seconds after end of asphyxia . . . . .	243	68
	45 seconds after end of asphyxia . . . . .	246	72
12:42 p.m.	Before sciatic stimulation (6 cm.) . . . . .	242	78
	During first 15 seconds stimulation . . . . .	245	100
	During next 20 seconds stimulation . . . . .	252	117
	Just after end of stimulation . . . . .	252	99
	20 seconds after end of stimulation . . . . .	250	86

	Rate	Pressure
12:50 p.m. Before asphyxia (80 seconds).....	250	72
During first 20 seconds asphyxia.....	251	82
During next 20 seconds asphyxia.....	251	66
During next 20 seconds asphyxia.....	252	64
Just after end of asphyxia.....	248	58

In one cat (452) we obtained practically no acceleration with asphyxia (maximum increase in heart rate 5 beats per minute) after removal of the adrenals, although before removal a maximum acceleration of 42 beats had been got in one observation. In the very first observation, however, with a somewhat shorter asphyxial period the acceleration was very slight (6 beats per minute). The blood pressure was exceedingly high after denervation of the heart (214 mm. of mercury). After removal of the adrenals by the abdominal route it sank to 114 mm. of mercury, and the pulse rate declined nearly 40 beats a minute.

*Condensed protocol.* Cat 452; old male; weight, 4.17 kgm. Under urethane (5.5 grams) cut vago-sympathetics and excised stellate ganglia

	Rate	Pressure
11:30 a.m. Before asphyxia (30 seconds).....	266	214
During first 15 seconds asphyxia.....	260	214
During next 15 seconds asphyxia.....	253	214
Just after end of asphyxia.....	270	207
15 seconds after end of asphyxia.....	272	215
35 seconds after end of asphyxia.....	265	206
11:35 a.m. Before asphyxia (45 seconds).....	258	190
During first 20 seconds asphyxia.....	258	207
During next 15 seconds asphyxia.....	256	220
During next 10 seconds asphyxia.....	271	205
Just after end of asphyxia.....	295	196
20 seconds after end of asphyxia.....	300	203
40 seconds after end of asphyxia.....	284	200
12:07 p.m. Excised both adrenals (extraperitoneally)		
12:08 p.m. Before asphyxia (45 seconds).....	222	114
During first 20 seconds asphyxia.....	222	118
During next 25 seconds asphyxia.....	219	121
Just after end of asphyxia.....	222	102
20 seconds after end of asphyxia.....	224	115
40 seconds after end of asphyxia.....	223	115
12:14 p.m. Before asphyxia (30 seconds).....	218	110
During first 15 seconds asphyxia.....	223	118
During next 15 seconds asphyxia.....	223	127
Just after end of asphyxia.....	220	110
20 seconds after end of asphyxia.....	220	120
35 seconds after end of asphyxia.....	223	120
12:18 to 12:35 p.m. Three more observations with asphyxia (60 to 90 seconds)		
gave a small drop in the rate during the asphyxia; the blood pressure had fallen to 84 at the end of the experiment.		

We do not think anybody who has studied our previous results will attribute the failure of the reaction in this experiment, after adrenalectomy, to elimination of the epinephrin output of the adrenals. But if by any chance a reader chooses to conclude, as apparently Doctor Cannon would do, that the drop of 36 beats a minute in the rate after removal of the glands is due to the absence of the epinephrin previously being given off from the adrenals (average 0.0002 mgm. per kgm. per minute in etherized cats) he will have difficulty in seeing why an increase in the output to many times this amount, with asphyxia, should be necessary to cause an acceleration of 42 beats a minute before the adrenalectomy, and why it should be impossible that a redistribution of the epinephrin without any increase in the rate of output might be responsible for the acceleration, even if asphyxia produces no change in the susceptibility of the heart to the action of epinephrin. The only possible conclusion, however, from our experiments is that whether the epinephrin takes any share in the acceleration associated with asphyxia or not, the reaction cannot be due solely to the adrenal epinephrin, and there is no evidence that epinephrin is concerned in it at all.

As has been pointed out in the previous paper (8), our proof that the increase in the heart rate caused by stimulation of the sciatic cannot be an index of an increased epinephrin output, does not entail the obligation to explain the acceleration, which is probably a complicated reaction. Still less do we feel bound to explain the asphyxial acceleration, which is possibly more complicated still. It is for Doctor Cannon, who brings forward the reaction as a quantitative test for changes in the epinephrin output, to exclude the other possible factors. We have suggested, however, (12) one possibility which must be controlled in so far as epinephrin may be a factor at all, namely, that asphyxia might render the heart more sensitive to such amounts of epinephrin as were being given off before the asphyxia was induced. Since we have shown that asphyxia in the absence of the adrenals can cause acceleration, it seems obvious that the accelerating action of a given dose of epinephrin might be reinforced by asphyxia. In that paper (12) we stated that it is not permissible to make quantitative comparisons on the effect of such a condition as asphyxia, using a test object like the heart well supplied with oxygen in one observation but asphyxiated in the comparison observation, without controlling any possible effect produced by the asphyxia upon the reactivity of the test object itself. There was every reason to point this out, as this factor is habitually

neglected by certain writers, who have worked with test objects *in situ*, although it must be taken account of before their results can be used at all.

Cannon now endeavors to control the possibility that asphyxia might alter the reactivity of the heart to one and the same dose of epinephrin, so that a reaction might be obtained simulating an increased output when the output was really unchanged. Although the question loses much of its interest, as a question in the technique of estimating epinephrin output, when it is known that the heart reaction relied upon by Cannon to demonstrate increased output is readily obtained in the absence of the adrenals, it may nevertheless be noted how Cannon takes account of the factor in question. He reproduces a curve taken while adrenalin, to the amount of 0.08 mgm. per minute, was being continuously injected into the veins of a cat. This is, for an average cat under ether, 200 times the mean rate of output of epinephrin under the conditions of our experiments, which according to Cannon may artificially increase the output to an "unpassable limit." The concentration of epinephrin in the blood passing through the coronary circulation must, therefore, have been much greater than could probably ever be sustained by the output from the adrenals, even if, according to Cannon, this were greatly increased by excitation of sensory nerves or asphyxia. The increase in the heart rate, however, was only 40 beats (from 132 to 172 per minute), an acceleration of the same order of magnitude as may be given by sciatic stimulation or asphyxia alone, without any artificial injection of adrenalin. Now, since the amount of adrenalin injected is, according to Cannon's own data, far greater than that liberated from the adrenals by asphyxia, it must be assumed that the acceleration was maximal before asphyxia was allowed to act. How can a possible change in the reactivity of the heart to epinephrin under the influence of asphyxia be demonstrated if the heart is already making its maximum effort in response to an enormous dose of injected adrenalin? The slowing of the heart, with the drop of pressure as the asphyxia continued, does not indicate that asphyxia of moderate duration renders the heart less sensitive to epinephrin, but is a very common phenomenon in asphyxia when no adrenalin has been injected, and when none can be coming from the adrenals. Epinephrin liberated at the ordinary rate may possibly enable the heart to resist better, and for a longer time, the depressing influence of asphyxia, and to respond better to the factors responsible for the acceleration either during the asphyxial period, or when respiration is resumed.

Another factor in the asphyxial acceleration, if epinephrin takes any share in it, may be, as already pointed out in the case of the acceleration caused by stimulation of sensory nerves (8), the redistribution of the blood associated with the vascular changes, which may result in a greater quantity of epinephrin per unit of time or a greater concentration of it being supplied to the coronary circulation, without any increase having occurred in the rate of output from the adrenals.

In another paper (12) we cited some evidence that certain reactions may be produced by epinephrin in this way, for instance, an experiment of v. Anrep (9) in which he shows that "if one splanchnic nerve is intact while the suprarenal on the other side is extirpated, stimulation of the splanchnic nerve on the side of the extirpated suprarenal may still cause constriction of a denervated limb. Only after the other splanchnic nerve is cut does the constriction disappear and the limb react passively to the change of blood pressure." His interpretation of this result is that "this is due to the fact that stimulation even of the peripheral end of the splanchnic excites a certain number of afferent nerves, so that there may be a reflex excitation of the suprarenal of the other side through the intact splanchnic nerve." There is no evidence that stimulation of the peripheral end of the splanchnic nerve can affect reflexly the rate of epinephrin secretion from the other adrenal and excellent evidence against it. The true explanation, we believe, is that so far as epinephrin is a factor in v. Anrep's reaction, the constriction seen under the circumstances described is due to the shunting through the denervated limb of more of the epinephrin being given off at the ordinary rate from the other adrenal. Cannon has suggested that in the cat (efferent) adrenin secretory fibers may occasionally pass from one splanchnic to the opposite adrenal, but he produces no proof of such a crossing. We have never seen evidence of any increase in the output of one adrenal when the opposite splanchnic was stimulated, or any evidence of diminished output when the opposite splanchnic was cut. Elliott's results on the protection of the epinephrin store from depletion in the adrenal whose nerves are cut, as compared with its fellow, could hardly have been what they were if both glands were innervated from each splanchnic. In the dog, v. Anrep could scarcely have failed to obtain the limb reaction in the experiment mentioned, after section of the opposite splanchnic, if there was a crossing of efferent fibers from the stimulated splanchnic to the other gland.

Redistribution of the blood owing to vasomotor changes is certainly not the only factor, if it is a factor, in the asphyxial acceleration, and in most of our experiments the rise of pressure produced by asphyxia after denervation of the heart was not great. Good accelerations were obtained with little or no increase of pressure, and the maximum acceleration was generally found when the pressure had fallen again, or, although rising after resumption of respiration, was still below the initial value. There was no obvious difference in this regard whether the adrenals had been excluded or not. Accordingly, such an experiment as Cannon illustrates in figure 7 of his paper (4) has no bearing on the question whether acceleration of the heart caused by asphyxia is due to increased epinephrin output. He denervated the heart, tied the limb and carotid arteries and severed the mesenteric nerves, and still got an increase in heart rate from 180 to 212 beats per minute after asphyxia, "with no previous noteworthy change in blood pressure." The pressure rose about 25 mm. of mercury after stopping the asphyxia,

and it was at this time that the maximum acceleration was counted. But how can this be a proof that asphyxia augments the output of epinephrin since equally large accelerations have been obtained by us in the absence of any epinephrin discharge?

As regards Cannon's method of demonstrating adrenal secretion by the rise of pressure caused by asphyxia after tying the carotid and limb arteries and "denervating the splanchnic area," it may be noted that the curve reproduced in figure 3 of his paper shows a slight elevation (less than 10 mm. of mercury) after the first minute of asphyxia, and a very large rise (at least 110 mm. of mercury) after resumption of respiration at the end of two minutes of asphyxia. He attributes both rises to the action of an increased output of epinephrin, but unfortunately does not state how much epinephrin would have been required to raise the pressure by 110 mm. of mercury. If such an enormous outpouring of epinephrin as would seem necessary to cause an effect of this kind were actually induced by asphyxia, it is impossible to see why we always missed it in collecting adrenal vein blood. Our operation was assuredly no more "severe" than Cannon's, the abdomen was opened by him also, and he states that the method yielded constant results so far as the belated influence of asphyxia was concerned, although "it was commonly disappointing as a means of demonstrating the early influence of asphyxia." If asphyxia can cause the liberation of so much epinephrin that a rise of pressure of this order of magnitude can be produced, it is also unintelligible that the careful experiments of Gley and Quinquaud (13) should not have revealed a striking difference in the effects of asphyxia on the blood pressure curve before and after eliminating the epinephrin output. They found that the curves were practically identical. Cannon's statement that after tying off the adrenals the rises of pressure did not occur, may mean nothing more than that the considerably lower blood pressure (during asphyxia it fell to little over 25 mm. of mercury) had so injured the heart that it could not respond to the resumption of respiration by such an increased action as, in the restricted circulation, would cause a marked rise of blood pressure, whereas, with the better circulation before exclusion of the adrenals it could do so.

These suggestions as to possible ways in which epinephrin may be a factor in the asphyxial acceleration of the denervated heart, without any increase in its rate of output having occurred, are not intended to imply that there is evidence that epinephrin takes any share in the acceleration. Our experiments show conclusively that it cannot be

the sole factor, if it is a factor at all, since marked accelerations can be obtained after exclusion or removal of the adrenals. As to the mechanism of the acceleration, we do not propose to enter further into a discussion of this reaction, in which direct effects of asphyxia upon the heart and indirect effects produced through the changed quality and quantity of the blood supplied to it may be intermingled. Our purpose was accomplished as soon as the statement that the reaction can be used to demonstrate an increased output of epinephrin during asphyxia was shown to be without foundation. Still less than in the case of the acceleration caused by sciatic stimulation can we imagine how such a reaction could be employed to measure quantitatively the rate at which epinephrin is given off by the adrenals.

Knowlton and Starling, working with the heart-lung preparation, have shown that the rate of the isolated mammalian heart is very sensitive to changes of temperature, although not altered by marked variations of arterial or venous pressure. But we have not included change of temperature of the blood coming to the heart during asphyxia or sensory stimulation, or change of temperature of the heart directly produced in asphyxia by stoppage of the heat loss through the respiratory tract, among the factors possibly concerned in the reaction because there is no evidence that under our experimental conditions this could have had any appreciable influence.

Cannon states that "the completely denervated heart can be used as an indicator of adrenal secretion in testing the influence of emotional excitement quite as well as in testing the influence of sensory stimulation and asphyxia." We have shown that it cannot be used at all in the case of sensory stimulation or asphyxia, and as, according to Cannon, it has the same value as a test for the influence of emotional excitement, we do not judge it necessary to discuss the matter here at any length.

Experiments on emotional excitement are necessarily less satisfactory than on sciatic stimulation or asphyxia because the animal must not be anesthetized. As a matter of fact, the evidence adduced by Cannon is extremely scanty and, in our opinion, will not bear examination. In one case, for instance, after removal of the left adrenal gland and section of the right splanchnic nerve in the thorax on the previous day an increase of 42 beats a minute was observed under excitement. In another case after a similar operation an increase of approximately 28 beats a minute was seen during excitement. These accelerations are of the same order of magnitude as those seen with both adrenals and their



nervous supply intact. How is it possible to consider a reaction as a quantitative test of the rate of epinephrin output which is given as well with one adrenal removed and the innervation of the other crippled as when both are normally discharging epinephrin? These animals had had many hours to recover from the effects of the anesthetic and operation and, therefore, gave a good heart reaction with excitement, a reaction which probably had little if anything to do with the assuredly diminished output of epinephrin from the remaining adrenal. That after removal of the remaining adrenal the reaction was not obtained when the animal was allowed to recover from etherization, is no proof that the failure of the reaction was due to the suppression of the residual epinephrin output of the gland, which was already largely denervated. The negative result is far more likely to have been due to the general condition of the animal just recovering from a second etherization and a second operation.

The only other experiment mentioned by Cannon is one in which excitement increased the heart rate from 217 to 255 beats per minute with the adrenals intact. Two days after this observation the adrenals were removed, of course under anesthesia, and about 5 hours thereafter excitement caused the rate to increase from 217 to 221 beats a minute. The conclusion is drawn that the difference in the result before and after the adrenalectomy is due to the loss of the epinephrin secretion. The possibility that the general condition of the animal after the adrenalectomy might be less favorable to eliciting a large acceleration is not considered. It must again be pointed out, as in the case of sciatic stimulation and asphyxia, that even if it had been proved that the failure of the reaction after removal of the adrenals was due to the absence of the adrenal epinephrin output and to this alone, the conclusion would not be justified that emotional excitement increases the rate at which epinephrin is liberated into the blood, until it was shown that the changes in the amount and concentration of the epinephrin passing through the coronary system, occasioned by the vascular effects of the excitement, were insufficient to account for the observed accelerations. We should have considered it a mere accident that the heart rate was the same (217 beats a minute) after removal of the adrenals as two days before. Cannon, however, believes that this "is an indication, and the first reliable one, that under quiet, peaceful conditions there is no adrenal secretion or a secretion so slight as not to affect the denervated heart, an extremely sensitive indicator." He argues that if there was a secretion of the magnitude observed by us there ought to have been a drop in the pulse rate when this secretion was eliminated. Doctor Cannon has not mentioned what the pulse rate was just before the adrenalectomy, so we do not know whether there was a decline in the rate or not after removal of the glands. We have, however, presented evidence both in the preceding paper (8) and in this, that other factors than the loss of epinephrin are concerned in the diminution of the pulse rate which usually follows removal of the adrenals and that sometimes that operation, or an equivalent interference with the epinephrin

output may not be followed by any diminution in the rate, while after exclusion of the adrenals a marked diminution may be caused by operative and other procedures associated with a fall of blood pressure.

*The catheter method.* Cannon sums up his work with the heart reaction by the declaration that "the results obtained with the isolated heart used as an indicator of adrenal secretion confirm in every respect the results obtained eight years ago by the catheter method." We have shown what kind of confirmation the isolated heart used as an indicator of adrenal secretion can lend to any other method, it could only render it suspect. Since, however, despite their confirmation by the isolated heart reaction, the results obtained by Cannon with the catheter method might be correct, we shall take this occasion to examine them on their merits in somewhat greater detail than has hitherto been done. This series of papers by Cannon and his co-workers deals with the influence of emotional excitement, asphyxia, stimulation of sensory nerves and some drugs upon the rate of epinephrin output. He collected blood from the inferior vena cava above the level of the adrenal veins by means of a catheter pushed up through the femoral vein. According to him, blood obtained from this region in cats in the absence of excitement, asphyxia, etc., does not cause inhibition of cat's intestine strips or rabbit's intestine segments, whereas blood obtained during or after the action of these factors causes marked inhibition, due to the outpouring of epinephrin. After emotional excitement lasting for 10 minutes or more, so much epinephrin has been or is being liberated that it can be detected in the general venous blood (femoral vein), after passing not only through the lungs but through the systemic capillaries. It may be remarked that if this is correct, it ought not infrequently to be possible to detect epinephrin in blood collected from patients or animals by puncture of a vein. For much excitement may attend even so small an operation. But no satisfactory evidence has ever been brought forward that detectable concentrations of epinephrin exist in the general venous blood. To account for the extraordinary output of epinephrin which would be necessary in order that it should be clearly detected in femoral vein blood, Cannon suggests that adrenalin liberated into the blood stimulates the adrenals to further increase the output. "Thus the more marked effect as time passes (see fig. 3) may be due not only to further excitement, but in part to an autogenous continuance of adrenal secretion. Thus also the persistence of the emotional state after the exciting object has disappeared can be explained. Indeed it was the lasting effect of excitement on digestive processes

which suggested this investigation." In support of this idea he appeals to a statement of Elliott's (14) that adrenalin itself causes depletion of the epinephrin store of the adrenals. Elliott himself later withdrew this statement (15), which in any case would not prove that adrenalin increased the output of epinephrin.

Cannon complains that "authors have written as if Cannon had been attempting to support the idea that emotional experiences were dependent upon circulating adrenin. Thus Stewart and Rogoff report, as if the matter had been questioned, that all signs of fright can be elicited by administering morphine to a cat with one adrenal removed and the other denervated." This subject is not referred to at all in the paper quoted by Cannon. In another paper (26), we are discussing the depletion of the epinephrin store produced by morphine, which Elliott (15) suggests is directly due to the fright caused by the drug. In this connection we say "That signs which might be interpreted as those of fright are present in cats under morphine is, of course, not doubtful. Whether this interpretation is correct might be difficult to decide, and does not concern us here. It is, however, of interest to note that epinephrin seems to have nothing to do with those signs. The signs of morphine fright can all be elicited by administering morphine to a cat in which one adrenal has been removed and the splanchnic supply of the other cut and in which accordingly no liberation of epinephrin through the splanchnics takes place." How can this be spoken of as "writing as if Cannon had been attempting," etc.? As a matter of fact, in that paper we were not discussing Cannon's psychological theories at all. We do not see that there was any impropriety in publishing observations showing that signs, which might be interpreted as those of fright, can be elicited by morphine in cats whose epinephrin output has been abolished or markedly diminished, even if we had been aware, which of course we could not be, that this detail in the pharmacology of the drug would seem self-evident to Cannon.

Cannon complains further that "Rogoff points out that the secretion of sufficient adrenin to produce symptoms of fright would be impossible, again as if any claim had been made that these symptoms were due to secreted adrenin." What Rogoff did say was: "In some of the cats (after removal of one adrenal and denervation of the other) it was found that the liberation of epinephrin from the adrenals was so interfered with by this operation that there could not have been one-thousandth of the normal liberation, if any epinephrin was being given off from the glands. These animals, nevertheless, responded readily to fright and other emotional disturbances with the usual symptoms of sympathetic excitation in the same manner as normal cats. Certainly, an outburst, through nervous influence, of epinephrin in such quantities as would be necessary to produce these symptoms would be impossible in these animals." Again, we fail to see the impropriety of quoting such a result, so long as it is true. We are glad that Doctor Cannon finds this result so obvious as not to be worth while mentioning. For it must then be obvious to him also that in these animals "the persistence of the emotional state after the exciting object has disappeared" cannot be explained as due to "continuance of adrenal secretion," whether "autogenous" or not.

Cannon seems to criticise our use of the term "denervated eye" for the eye after removal of the corresponding superior cervical ganglion, and says that he and de la Paz "tried the denervated eye method of testing for adrenal secretion but could not persuade themselves that an eye still innervated by the third cranial nerve was really denervated." It is surely unnecessary to state that when we use the term "denervated eye" in this sense we do so for convenience, following the practice of other writers, for instance, Elliott (15). We frequently speak of "the (denervated) eye reactions of Meltzer," or simply "the eye reactions," or of "the iris sensitized by previous removal of the superior cervical ganglion." Cannon himself uses the terms "excited" blood and "quiet" blood for convenience, and quite properly, although he will not maintain that they are literally correct. We did not imagine that any reader would suppose that we were not aware that the oculomotor nerve supplies the eye. We agree with Cannon that "the prompt dilatation of the pupil in a paroxysm of rage," in cats whose epinephrin output has been interfered with by removal of one adrenal and section of the nerves of the other has nothing to do with epinephrin, but is due to inhibition of the pupillo-constrictor mechanism. We have attributed the dilatation caused by stimulation of sensory nerves, including the paradoxical reaction, which can be obtained after removal of the adrenals (8), to the same mechanism. Long ago, it was stated by one of us (27) that sexual excitation caused very marked dilatation of the pupils, in dogs of both sexes, after division of both vago-sympathetic nerves (without removal of the superior cervical ganglion) by central inhibition of the constrictor pupillae through the oculomotor.

Apparently it is necessary to point out again, as has been done repeatedly before (1), (12), that when we employ the eye reactions to test for epinephrin, we eliminate factors which can affect the eye through the nervous system, by collecting the adrenal blood in a cava pocket and only releasing it, as far as possible, after these factors have ceased to act.

Cannon, on the basis of his experiments with the catheter method, concluded that emotional excitement, asphyxia and sensory stimulation increase the rate of output of epinephrin. Nicotine does the same, whereas urethane has no effect. Our own experiments with asphyxia and sensory stimulation, made by methods which permitted the rate of output of epinephrin to be estimated, did not support Cannon's conclusion, yielding no evidence of sensibly increased output. Such negative results must always yield to positive results obtained by a better method, and this has been emphasized by us. Cannon's catheter method, however, far from being a better method than those employed by us, is not a method by which the rate of output of epinephrin can be estimated at all. As a matter of fact, his papers do not contain a single estimate of the output of epinephrin before, during or after the action of the factors which he is studying. And it is clearly impossible that they should contain such data. The most that the method could yield would be the epinephrin concentration in the cava blood above the

adrenal level. Without knowing the amount of blood with this epinephrin concentration passing the point of collection per unit of time, the output of epinephrin could not be calculated. But even the epinephrin concentration was not determined by Cannon. He gives not one estimate of the concentration, or any information as to the minimum concentration which his strips or segments could have detected. In the absence of information of this kind it is impossible to deduce any conclusion from the few curves reproduced. What, for instance, was the concentration of epinephrin in the femoral vein blood after emotional excitement? If we knew whether it was 1:1,000,000 or 1:10,000,000 we might be able to check the probability of the statement by calculating the concentration which must have existed in the blood of the adrenal veins, and seeing whether anyone has ever observed so high a concentration. If the minimum concentration clearly detectable by the segments and the concentration actually found in the cava blood during asphyxia, etc., had been determined, there would be some possibility of deciding whether the observed changes in concentration could be due to alterations in the rate of blood flow in the cava without any change having occurred in the rate of output of epinephrin.

We have suggested that vasoconstriction, especially in the splanchnic area, which may be assumed to accompany the rise of blood pressure associated with stimulation of the central end of the sciatic or with asphyxia, will cause slowing of the blood flow in the inferior cava. Doctor Cannon seeks to invalidate this suggestion by the statement that an increased arterial blood pressure causes an increased blood flow through the *adrenals*. Had this argument not been repeated and emphasized in his paper we should have considered it a slip. Certainly the adrenal flow increases when the arterial pressure rises. But as the blood from the adrenals is only a small fraction (perhaps  $\frac{1}{100}$  to  $\frac{1}{1000}$ ) of the blood passing along the inferior cava this, of course, has no sensible influence upon the rate of cava flow. Cannon further argues that when the arterial pressure is raised by splanchnic vasoconstriction the blood flow in the inferior cava may be increased. We had supposed it was universally admitted that vasoconstriction renders the passage of the blood through a vascular area more difficult, and that the arterial blood pressure rises for this very reason when an important area, like the splanchnic area, is constricted. We are not concerned here with compensatory reactions which may occur in other areas, leading, for example, as Edwards (16) has shown, to increased flow in the superior cava. It is precisely because less blood is passing through the vasoconstricted areas that more blood can pass through areas which are not constricted.

Vasomotor effects are not the only factors which may influence the rate of the blood flow in the inferior vena cava under the conditions of Cannon's experiments. The respiratory movements and, therefore, the intrathoracic pressure are affected by sensory stimulation, emotional disturbance and very grossly by

asphyxia. It is not possible to produce such changes without markedly affecting the pressure in the great veins. The region of the cava from which a sample of blood is assumed to be drawn off through the catheter is right up against the diaphragm. How can the control sample be obtained under the same conditions, except for the hypothetical stimulation of the adrenal secretory mechanism, as a sample taken when the animal is gasping in asphyxia? In asphyxia the right heart and great veins become engorged with blood. The blood in the cava must, therefore, be dammed back and if the adrenal goes on steadily secreting epinephrin the concentration in the upper segment of the cava must increase without any change having occurred in the rate of output. If the animal, in struggling or gasping, markedly increases the intraabdominal pressure the cava might be more or less obstructed by flattening of its walls upon the catheter, especially above its upper end.

There are still other ways in which the blood flow may be altered through effects produced upon the heart by the factors studied by Cannon. Yet in none of his papers has he indicated that there is any necessity to take into account possible changes in the rate of blood flow. It is the same with the question whether massage of the glands, particularly of the right adrenal, may not be produced by the catheter during the voluntary movements executed by the animal when frightened or the movements of the diaphragm in asphyxia. The right adrenal lies close against the cava, separated from the catheter by little more than the thin wall of the vein, and it is well known that massage causes liberation of epinephrin from the adrenals. All these possibilities ought to be controlled.

In referring to our negative results with the catheter method Cannon states, what could not be concluded from anything in his previous papers, that "the method is difficult and exacting, and that not until after some experience with it did it begin to yield us positive results." He rebuts our suggestion that some of his positive results might be due to a fortunate location of the eye of the catheter with reference to the orifices of the adrenal veins by saying "that it seems to have been made with disregard for the care exercised in making control observations under precisely the same conditions before and after stimulation." This is beside the point. There is no particular difficulty about inserting a fine catheter into the cava, so that in successive observations a string tied on the catheter is at the same level of the femoral vein, while the eye of the catheter is judged to be above the level of the adrenal veins. It is easy to turn the catheter so that a mark on its circumference always occupies the same position. But the exact relation of the eye of the catheter to the orifices of the adrenal veins or to the orifice of one of the veins, e.g., the right, since they do not enter the cava at the same level, can never be known till the abdomen is opened, if then, nor can it be assured that this relation will always remain precisely the same during such movements as the animal may make, even when securely tied down, during excitement caused by a barking dog or during stimulation of sensory nerves or asphyxia.

The few curves published by Cannon, are not at all convincing. In all the work with the catheter method he has contented himself with one or two comparative tests of the bloods. We have laid stress on the necessity of repeated comparison. His method of emptying the cylinder by sucking out one liquid

before the application of the next is also liable to introduce error, as we have previously pointed out (18) and illustrated by curves. Not only may the tracing be deformed at the critical moment, but the strip or segment is suddenly exposed and then suddenly immersed in liquid. He refers to a paper by three young medical men (17) as having confirmed his results on emotional excitement. But this paper contains not a single tracing or protocol, not one estimation of the epinephrin concentration in the blood or of the concentration which it would have been possible for the segments to detect. There is internal evidence that any reaction obtained could not have been due to epinephrin. For example, they state that when blood collected from the cava above the level of the adrenals, during the action of factors which increase epinephrin output, was allowed to stand for 25 minutes, it no longer caused inhibition of the rabbit's intestine segment, "and epinephrin is the only oxidizable substance in the body that produces inhibition of the intestine which can be oxidized in 25 minutes." Now, a sample of blood, known to contain epinephrin in amount sufficient to give a decided inhibition of the intestine, does not become ineffective merely by standing for 25 minutes. At one place it is stated that morphia prevents the epinephrin reaction from being given by the cava blood under the influence of Witte's peptone, and in another place that morphia distinctly diminishes the normal output of epinephrin, as tested in blood taken directly from the adrenal vein. In still another place the statement occurs that "substances with which we have experimented consisted of the toxins of gonococci, of streptococci, of staphylococci, of colon bacilli, of tetanus bacilli, of diphtheria, foreign proteids, indol and skatol, leucin, creatin, feces extract, strychnin, *morphia*, carbolic acid, Witte's peptone, sheep serum, ox serum, kitten serum. All of these have seemed to cause adrenal activation, and from their use 61 out of 66 consistent experiments can be reported . . . . Narcotics and anesthetics caused no increased output of adrenalin . . . . The various agents which caused increased epinephrin output, or which diminished it, are either neutral as to motion and fever or diminish them. . . . There was no epinephrin reaction in any animal (subjected to intense emotion, rage and fear) in which bilateral division of the splanchnic nerves existed . . . . Of great significance is the fact that all agents that produced an increase of adrenalin output cause also a hyperchromatism of the brain followed by exhaustion. It mattered not whether this stimulating agent was a physical exertion in running, in fighting or in convulsions; or whether it was the emotion of fear, or of anger, or a reaction to anaphylaxis, to toxins, to indol, skatol, foreign proteids, or strychnin, in every case hyperchromatism and increase of adrenalin output went hand in hand." All this without tracings, without any sample of the great mass of experimental details which would be necessary to establish so many conclusions. If it is to be a matter of taking anybody's word for a physiological result, with all due respect to these writers, whom we know to be very competent men in their own subjects, we should much sooner take the word of an accomplished physiologist like Doctor Cannon himself.

In Cannon's paper on emotional stimulation the first figure shows that adrenal vein blood, obtained under ether, when substituted for inactive blood caused relaxation of the intestine strips, whereas blood from the renal vein did not give this effect. The most which could be deduced from this is that adrenal vein blood has a demonstrable content of epinephrin, a proposition which is generally

accepted. The second figure shows that when so-called excited blood (i.e., blood removed through the catheter from above the adrenal level during excitement) was substituted for Ringer's solution, the tone of the intestine strip was markedly increased. It remained increased for about 4½ minutes, and then the strip relaxed and the beats disappeared. Cannon interprets this extremely belated relaxation as an epinephrin reaction. This is contrary to all our experience. An epinephrin relaxation is produced immediately or not at all. A relaxation occurring 4 or 5 minutes after the application of a blood cannot be accepted as a positive reaction for epinephrin. A second application of the excited blood, this time replacing so-called "quiet" blood caused a prompt relaxation. The only conclusion which can be drawn from this figure is that two inconsistent observations with a given sample of blood on such an object as an intestine strip or segment cannot be accepted as evidence that the blood from the inferior cava, drawn during excitement, gave a positive epinephrin reaction. If an undoubted positive reaction had been obtained, this, as already pointed out, would yield no information as to whether the rate of output of epinephrin had been increased by the excitement or not.

The third figure professes to demonstrate that with the prolongation of excitement the effect on the epinephrin secretion goes on increasing. This is done by comparing a record of the strip in defibrinated blood drawn from the cava after 11 minutes excitement with a record in the *serum* of blood obtained after 15 minutes of excitement. In the defibrinated blood the strip retains the increase of tone caused by substituting the blood for Ringer's solution, for about 2 minutes, and then begins to relax. In the serum the strip relaxes more promptly. If the two samples of blood had the same concentration of epinephrin, the serum would necessarily give a stronger reaction than the blood since practically all the epinephrin is in the serum (19). After the initial increase of tone has lasted 2 or 3 minutes some relaxation occurs also with "quiet" serum, and the beats become slower. No data are given by which it would be possible to judge how great the difference in epinephrin concentration in the different samples was.

The fourth figure is intended to show that after removal of the adrenal glands, blood from the cava of an excited animal above the adrenal level gave no inhibition of the intestine strip when substituted for Ringer's solution. As there is no question that epinephrin exists in the upper cava blood, so long as the adrenals are intact, and must disappear when the adrenals are excised, nobody will dispute that a test object, if sensitive enough, will give a positive reaction in the first case and not in the second. In the legend of this figure, it is stated that "the strip later proved sensitive to adrenalin in blood in the ratio 1:1,000,000," and in the fifth figure tracings are given showing the inhibition produced by blood with 1:1,000,000, 1:2,000,000 and 1:3,000,000 adrenalin when substituted for "quiet" blood. The 1:3,000,000 adrenalin blood gives a reaction much smaller, and the 1:2,000,000 adrenalin blood a reaction probably smaller than those figured by Cannon as given by "excited" blood. He does not state specifically that these curves were obtained from strips used to test any of the "excited" bloods whose records are reproduced, and, as has been pointed out, it is unfortunate that he did not assay the concentrations of epinephrin which he believed these bloods to contain. But there would seem to be no point in reproducing tracings showing the inhibition of intestine strips given by such concentrations



of added adrenalin, unless they had a bearing upon the magnitude of the reactions given by the cava bloods. Redfield (20) who worked with Cannon's method, and partly under his direction, has stated that he succeeded in modifying the method so as to render it capable of detecting 1:10,000,000 adrenalin. Redfield obtained such variable results in experiments, in which he endeavored to detect epinephrin in the blood (of the horned toad) during emotional excitement by means of the intestine reaction, that he says "no weight can be placed upon the experiments."

It seems pretty clear that in Cannon's hands the method was not very sensitive, and that such inhibitions as he figures, if due to epinephrin, must have corresponded to epinephrin concentrations in the "excited" cava blood of the order of magnitude of 1:1,000,000 or 1:2,000,000. Now, these concentrations are double the average concentrations found in adrenal vein blood, with the average normal arterial pressure under the conditions of our experiments, and would represent 100 to 200 times our average normal output. Cannon in his last paper (4) has stated that with sciatic stimulation he can increase the output to 5 to 25 times our "normal" output. But that apparently is nothing to what can be obtained when a cat is frightened by a dog. For us, such concentrations in the cava blood (corresponding perhaps to 1:10,000 in the adrenal vein blood) are so improbable that we are unable to accept them on such evidence as Cannon has furnished. If anything like such concentrations of epinephrin could exist in the cava blood with ordinary rates of blood flow, it would be impossible for us to miss them with the method of assay which we employ.

In the sixth figure a tracing is given, showing that "active" blood applied to a strip caused relaxation after 2 minutes. The same blood, after oxygen was bubbled through it for 3 hours, when applied to a *fresh* strip is stated to have failed to cause relaxation. If the reactivity of the new strip was the same as that of the other (which was not controlled, but which must always be controlled if reliable results are to be obtained with such test objects) this would indicate that an oxidizable substance (epinephrin) was responsible for the original inhibition.

Cannon cites Redfield (20) as having "reported that in the horned toad nervous excitement causes a contraction of the melanophores in the denervated skin, a reaction which does not occur after the removal of the adrenal glands." He adduces this as a proof that in this animal emotional excitement increases the epinephrin output. As a matter of fact, Redfield found that in the great majority of his experiments removal of the adrenals "does not check the contraction of the melanophores." Out of a large number of animals he only succeeded in finding two in which after adrenalectomy (involving the opening of the body cavity, removal of the gonads and a portion of the genital ducts and tying off the posterior cava) contraction of the melanophore pigment could not be obtained "when the mouth was stimulated by a weak faradic current." There is no proof that in these two exceptional cases it was the loss of the epinephrin output of the adrenals and not some other factors, such as the general deterioration of the animal caused by the operation, which was responsible for the negative result. However, his experiments with denervated skin and temporary ligation of the blood supply of portions of skin suggest strongly that epinephrin given off from the adrenals is a factor in contraction of the pigment. But we do not

find any proof that the effect is due to an increased epinephrin output. An experiment is quoted to show that blood from an excited horned toad causes a contraction of the melanophore pigment of an unexcited animal in the neighborhood of the point of injection, whereas blood obtained from an animal after destruction of the portion of the thoracic cord, whose integrity is essential to the production of contraction of the melanophore pigment through noxious stimuli, has no such effect. All that this experiment could indicate would be that the effect of the blood from the animal excited with intact cord was due to epinephrin. The experiment throws no light upon the question whether excitement can increase the epinephrin output. For if the injury to the thoracic cord prevents the contraction of the melanophore pigment associated with stimulation, by interfering with the ordinary output of epinephrin, the blood of the excited animal would necessarily contain more epinephrin than that of the control, without any increase in the rate of output having occurred in the former. We have shown (21), (22) that in mammals the upper part of the thoracic cord is intimately related to epinephrin secretion. The possibility must be considered that in this animal, on account of slower circulation, less active metabolism, and perhaps lower temperature of the blood and tissues, a greater concentration of epinephrin may exist in the general blood than in mammals. The great concentration found by Abel and Macht (23) in the secretion of the so-called parotid gland of a tropical toad suggests that care may be necessary in applying results obtained in such animals to mammals. The above remarks are in no way intended as a criticism of Redfield's paper, which seems to us a most interesting and suggestive piece of work.

In Cannon's paper on the effects of asphyxia, hyperpnoea and sensory stimulation on adrenal secretion there are three figures. Rabbit's intestine segments were employed for the tests. The first figure shows a marked effect of disturbance of the curve by sucking out the liquid. If any weight can be laid on a curve of this kind, the "normal" vena cava blood (i.e., from above the adrenal level in the absence of asphyxia) probably caused some inhibition and the cava blood from the same level after asphyxia, a greater inhibition. But how is it possible to determine whether the difference in epinephrin concentration between the two cava specimens is great or small?

The second figure purports to demonstrate the failure of hyperpnoea to increase adrenal secretion. "The chest was opened and the chest walls held apart while the lungs were inflated in rapid repetition by means of bellows. The air forced into the lungs was permitted to escape quickly through an opening in the trachea." The curve shows that when vena cava blood taken from above the adrenals with ordinary breathing is substituted for Ringer's solution, there is some increase of tone, but the beats continue. When vena cava blood drawn from above the adrenals after the application of hyperpnoea is substituted for the previous sample of cava blood no notable change occurs in the tracing. The conclusion is "that hyperpnoea, to a degree resulting in acapnia is not attended by increased adrenal secretion." This seems to us extremely probable, but what the tracing indicates is simply that no noteworthy change has occurred in the concentration of epinephrin in the cava blood, within the limits of sensitivity of the segment. It is obvious that the conditions which in the asphyxia experiments may lead to slowing of the blood flow in the cava are absent here.

The chest being widely opened, changes in the intrathoracic pressure and in the movements of the diaphragm are eliminated. The situation as regards vasomotor and cardiac changes, especially splanchnic vasoconstriction, is likewise quite different. Since Cannon has here performed a considerable operation in an anesthetized animal, and since, according to him, the trauma and anesthesia in our experiments increase the "ordinary" output of epinephrin so much that it may be impossible for asphyxia or sensory stimulation to cause any further increase, the question would seem pertinent why he obtains in this experiment a totally negative reaction for epinephrin in the cava blood taken above the adrenal level. Our explanation is that the small adrenal output is too much diluted by indifferent blood in the cava to be easily detected.

The third figure in this paper purports to show that sensory stimulation increases adrenal secretion. What it does show is that when cava blood from above the adrenal level, obtained during sciatic stimulation, is substituted for cava blood obtained without sciatic stimulation, the writing point descends abruptly. A large part of the descent appears to be due simply to the fact that when the latter blood was sucked out of the cylinder the weight of the segment, coming on the lever, raised the point considerably. When the cylinder was again filled up with blood, the weight of the segment was nearly neutralized and the writing point necessarily descended. The true effect of the inhibition of the segment is, therefore, considerably exaggerated. All that can possibly be deduced from the tracing is that the epinephrin concentration in the sample collected during sciatic stimulation was greater than in the other. No assay of the concentration was made and, therefore, it is impossible to know what the difference was. It might have been a very moderate difference or a very great one. Inspection of such a curve can yield no information as to this. Also, it is not stated how many times the result figured was confirmed on the same samples of blood.

In his paper on the effect of nicotine on adrenal secretion Cannon has published three figures. The first shows that cava blood from above the adrenal level, secured before nicotine injection, when substituted for Ringer's solution caused an increase of tone of an intestine strip. When cava blood from the same level, obtained after injection of nicotine, was substituted for the other cava blood, there was a prompt and large diminution of tone. The conclusion is drawn that the adrenal secretion was increased by the nicotine. The most, however, which can be deduced from the tracing is that the concentration of epinephrin in the second specimen is greater than that in the first. If the blood flow is slowed, as would be the case after the doses of nicotine employed, an increased epinephrin concentration would be consistent, not only with an unchanged, but even with a diminished output. We have already pointed out (24) how completely Cannon, owing to the faulty method employed, has missed the details of the nicotine action on the epinephrin output, the most outstanding and most durable effect being a depression of the output.

It is stated in the text that the several specimens of blood were tested for their content of epinephrin. But not one tracing illustrating the assay is reproduced, nor is a single concentration given, although the statement is made that "the characteristic inhibition of the rhythmic contractions of the muscle, even when it has been for some time removed from the body, occurs at a dilution of adrenalin 1:2,000,000 in defibrinated blood." If it is implied that this is the order of

magnitude of the concentrations which cause inhibitions like that displayed in the figure under discussion, we can only repeat that it is in the highest degree improbable that such concentrations can be obtained in a fair sample of the cava blood above the adrenal level, unless there is an extreme slowing of the cava flow. Our own work on nicotine shows that at the time Cannon collected his samples the initial brief stimulating action of nicotine on the epinephrin output must have long since passed into the stage of depression. The effect of the first cava sample when substituted for Ringer's solution cannot, of course, be compared with that of the second cava sample when substituted for the first. To assay the two specimens they should be caused separately to displace the same sample of indifferent blood free from epinephrin. The displacement of one sample of epinephrin-containing blood by another sample of epinephrin-containing blood is not usually satisfactory for purposes of assay. For *a*, the first sample has already produced an epinephrin effect upon the test object which may render the inhibition, caused by the second sample when it displaces the first, different from what it would otherwise be. *b*, The epinephrin content of the first sample in contact with the test object, especially if left for a considerable time in contact with it, is diminished, so that the content of the second sample when now caused to displace the first may be exaggerated.

The second figure in Cannon's nicotine paper shows that the quantity of nicotine employed could not have accounted for the inhibition of the intestine strip produced by the cava blood after nicotine injection. This is unquestionably correct. The third figure demonstrates that cava blood obtained from an animal after removal of the adrenals and after administration of nicotine, does not cause an inhibition of the intestine strip. This is no doubt perfectly true, but it simply indicates that in the absence of the adrenals no epinephrin detectable by the method employed exists in the cava blood. It would be much more to the purpose to state what were the concentrations of epinephrin in the cava blood and the rates of cava blood flow before and after injection of nicotine. But the reader searches in vain for this essential information. That the intestine strip was inhibited by the addition of one drop of adrenalin 1:1000 to a cylinder containing 2.5 cc. of blood (which would make a concentration of about 1:35,000 or 1:40,000) is no doubt correct. But what possible relation has a huge concentration like this to anything which can occur in the cava blood?

#### SUMMARY

1. The acceleration of the heart induced by asphyxia after division of the vagi and excision of the stellate ganglia cannot be taken as an index of increased epinephrin output from the adrenals, as assumed by Cannon. For it may be obtained and may not be diminished in amount after the adrenal veins have been ligated, after the adrenals have been completely isolated from the circulation, and after the adrenals have been excised.

2. Toward the end of an experiment, after repeated periods of asphyxia and when the general condition of the animal, including the

circulation, has deteriorated the reaction is apt to become much less marked or to fail altogether. An operation like adrenalectomy, when it accelerates the deterioration of the animal and of the heart by lowering the blood pressure decidedly, may sometimes seem to be responsible for the failure of the asphyxial acceleration. But there is evidence that this is not due to any specific effect of the adrenalectomy (loss of epinephrin output) but to the general effect of the operation. When asphyxia fails to cause a decided acceleration it is often seen that some slowing has occurred during the asphyxial period, succeeded by a quickening of the beat on resumption of respiration. It is clear that it will depend upon the state of the heart whether this subsequent acceleration will bring the heart back to, or nearly to, its original rate or cause it to pass considerably beyond the original rate. Whether an acceleration of some magnitude, as compared with the rate before asphyxia, can actually be counted on the trace is then more or less of an accident, depending upon whether the deteriorated heart comes through the period of asphyxia in such a condition as to permit it to attain a rate considerably in excess of the initial rate.

3. There is no good evidence that asphyxia causes an augmentation of the rate of epinephrin output by a direct action upon the cells of the adrenal medulla.

4. The acceleration caused by asphyxia, like the acceleration caused by stimulation of the central end of the sciatic, is not interfered with by opening the abdomen. There is no evidence that the operation necessary for collecting adrenal vein blood for direct assay of its epinephrin content would render it impossible to detect an increase in the epinephrin output, if this were caused by asphyxia or stimulation of sensory nerves.

5. No real evidence has been adduced that the epinephrin output, measured by those observers who have adopted the fundamentally correct method of collecting the blood and assaying it on suitable test objects, is an output artificially increased by anesthesia and trauma. On the contrary, the remarkably narrow range of the output in different animals, under different anesthetics and with the different operations strongly suggests that it is a physiological output already going on, not initiated and probably not much modified, but merely unveiled by the experimental procedures necessary for its measurement.

6. As a method of estimating changes in the rate of output of epinephrin from the adrenals, the catheter method is defective in principle. For at best all that could be measured by it would be changes in the

epinephrin concentration in the blood of the inferior cava above the level of the adrenals. Changes in the rate of flow of the blood are not taken account of. The quantity of epinephrin passing along the inferior cava to the heart per unit of time cannot be estimated, nor the changes, if any, produced in this quantity by the conditions studied. We have pointed out that in the cases in which Cannon claims to have obtained evidence of an increased output of epinephrin, all he can possibly have observed is an increased concentration in the cava blood, and that a slowing of the cava flow would cause an increase in the concentration, if no change whatever had occurred in the rate of output. We have suggested certain factors, associated with all the conditions studied by him, which might cause such changes in the cava flow as would tend to increase the epinephrin concentration, even in the absence of an increased rate of output. However, the complete lack of assays of the concentrations of epinephrin supposed to have been present, and of estimates of the concentration which could have been detected by each segment or strip employed, renders it impossible to determine to what extent positive reactions, when obtained, were actually due to epinephrin. It cannot be assumed that by sucking blood from a catheter opening into the cava immediately below the diaphragm, a region where the blood flow is necessarily less steady than anywhere else, owing to the influence of changes in the intrathoracic pressure, the same aliquot part of adrenal blood will be drawn off in successive samples without regard to the gross effects upon the respiration produced by such conditions, e.g., as asphyxia. Such mechanical changes may alter the proportion of adrenal blood to the much greater quantity of blood from other sources drawn off through the catheter. Our experience with the catheter method fully justifies the criticism of Richards and Wood (25) that the method is "highly faulty in that the blood from the suprarenals is diluted with that from all of the structures whose veins enter the cava below the suprarenals."

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# THE EFFECT OF SPLENECTOMY UPON GROWTH IN THE YOUNG

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## INTRODUCTION

With but few exceptions the removal of the spleen has been done on adult animals. Might it be that splenectomy in the young would exhibit some change not yet observed? It is known that removal of thyroid glands in young rabbits is followed by much more striking changes (experimental cretinism) than are manifested by removal of these glands in the adult animal.

## LITERATURE

Wolferth (1) reports blood changes in rats splenectomized at thirty days of age, comparable to those changes in the blood of adult splenectomized rats. There is a slight to moderate anemia persisting for three or four weeks with recovery in a few months. Accompanying this is a slight leucocytosis and an increased resistance to hypotonic sodium chloride solutions and hemolytic agents.

Tachigara and Takayi (2) find that in general the effects of splenectomy in newborn dogs are the same as in adult animals. The most marked result is an increase in the number of red corpuscles. The younger the dog at the time of operation, the greater is this increase and the earlier does it appear. Their protocols likewise show that the splenectomized puppies operated from one day to two months make gains in weight that are nearly parallel to normal animals through periods of six to eight months. They also observed that the spleen of young animals weighs more in proportion to the body weight than it does in older animals. This is a factor that has been reported clinically by Gross (3) in a statistical study of the spleen.

On two male dogs from the same litter, one splenectomized and one a laparotomy control, operated by Doctor Luckhardt at four weeks of



age, the weight curves were found to differ. The splenectomized animal gained in weight but not in the same proportion as did the control. These dogs suggested a difference in metabolism as judged from the weight curves. These dogs were very kindly given to me by Doctor Luckhardt<sup>1</sup> at four weeks after operation for further observations. (Vide infra).

Removal of the spleen in adult animals presents results that differ, yet suggest that the spleen may have an influence in metabolism. Richet (4) reports in his metabolism experiments that splenectomized dogs must eat a much greater quantity to maintain the same nutrition as normal dogs. Spitta and Mayo (5) report an increase in weight following splenectomy. Paton (6) concluded that the spleen had no influence upon metabolism. Goldschmidt and Pearce (7) although differing from Paton on his blood studies, concluded that there was no effect upon metabolism, but observed that splenectomized dogs had a tendency to become obese. From observations in this laboratory upon adult splenectomized dogs it has been noticed that for a time following splenectomy dogs were emaciated but later became quite fat.

The possibility that the spleen assumed a greater functional importance in the young than in the adult animal as suggested by the proportionate differences in the weight of the organ at these two periods of life, as well as the contradictory results in metabolism of splenectomized dogs, led to this question: What effect has the removal of the spleen upon the metabolism of young animals as judged from the growth and weight curves?

#### METHODS

*Operative procedure.* In this work rats and rabbits have been the principal animals upon which the work has been done. Observations were made upon the dogs already mentioned and upon two series of kittens. Unfortunately distemper and accidents prevented further data on kittens and puppies.

The rats and rabbits were operated at fourteen days of age under ether anesthesia. Of the cats recorded, one series was operated at fourteen days and the other at seven weeks. It was found inadvisable to operate earlier than two weeks, on account of the extreme delicacy and softness of the young tissues.

<sup>1</sup>The work reported in this paper was done upon the suggestion of Dr. A. B. Luckhardt whose kindly interest and constant help has been most gratefully appreciated by me.

Removal of the spleen was made through an incision in the left rectus. Curved forceps were used in removing the spleen so that the incision might be small. In rats no ligatures were placed upon the splenic vessels but slight compression by forceps prevented any hemorrhage. In the rabbits, kittens and puppies silk ligatures were placed upon the splenic vessels. A frog board makes an excellent operating table for the rats.

That the operative procedure might be nearly comparable in the splenectomized and control animals, the controls were kept under either the same length of time and the viscera were manipulated for a short time. In rats a few animals were observed which were unoperated controls. This was done as a check upon the effects of the operative procedure.

*Diet.* The diet of rats consisted of oats, bread, milk, carrots and, when obtainable, lettuce or cabbage. At intervals of two or three weeks raw meat was fed. The diet of rabbits was that of the rats save that bread, milk and meat were not fed.

The diet of the kittens was table scraps, bread and milk, and meat. The dogs were fed a diet similar to cats without the addition of milk.

*Weighing.* The animals were weighed just before operation and at weekly intervals prior to the time of feeding.

#### RESULTS

That individual variations might not lead to prejudiced or erroneous conclusions, composite weight curves were made of the rats and the rabbits.

*Rats.* In figure 1 is given the composite weight growth curve of five male operated controls and eight male splenectomized rats over a period of twenty-three weeks. Of these rats there is in table 1 a record of one typical set. In this table there is also a record of one unoperated control. This record is typical of all unoperated animals. The composite curves show that there is very little difference between the splenectomized and operated animal controls, the difference never exceeding 10 grams and this in favor of the splenectomized animals.

Figure 2 represents the composite weight curves of nine female splenectomized and eight female operated control rats over a period of twenty-three weeks. A typical set from this series is presented in table 2. As in figure 1, it may be seen that there is no appreciable difference in the weight curves. Here again the splenectomized ani-

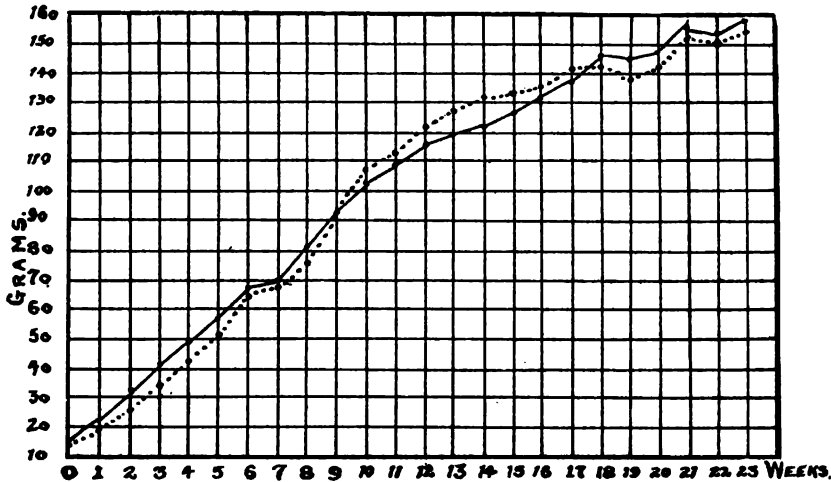


Fig. 1. Composite weight curve of male rats. Five operated controls——; eight splenectomized..... Operated at 0 when fourteen days of age and carried through a period of twenty-three weeks.

TABLE I  
Male rats

Three rats: no. 12, an unoperated control; no. 13, a splenectomized animal; no. 14, an operated control. The latter two animals were operated at fourteen days of age, and have their weights included in those of the composite curves of figure 1. The weights of the unoperated control were begun at fourteen days of age, and with those weights of the other two animals were carried through a period of twenty-four weeks.

				DATE OF OPERATION						
				2/4/19	2/12	2/18	2/25	3/4	3/11	
Rat 12. Unoperated control .....				17	24	30	37	44	49	
Rat 13. Splenectomized .....				17	24	31	45	58	68	
Rat 14. Operated control .....				18	25	34.5	50	59	66	
	3/18	3/25	4/1	4/8	4/15	4/22	4/29	5/6	5/13	
Rat 12.....	56	62	70	82.5	98	109	117	120	127	
Rat 13.....	74	85	92	112	137	144	147	154	160	
Rat 14.....	72	83	92	110	130	137	145	153	162	
	5/20	5/27	6/3	6/10	6/17	6/24	7/1	7/8	7/15	
Rat 12.....	129	134	140	137	137	139	150	148	145	
Rat 13.....	155	162	163	169	162	164	185	178	183	
Rat 14.....	162	163	165	174	172	173	190	178	184	

All weights are in grams.

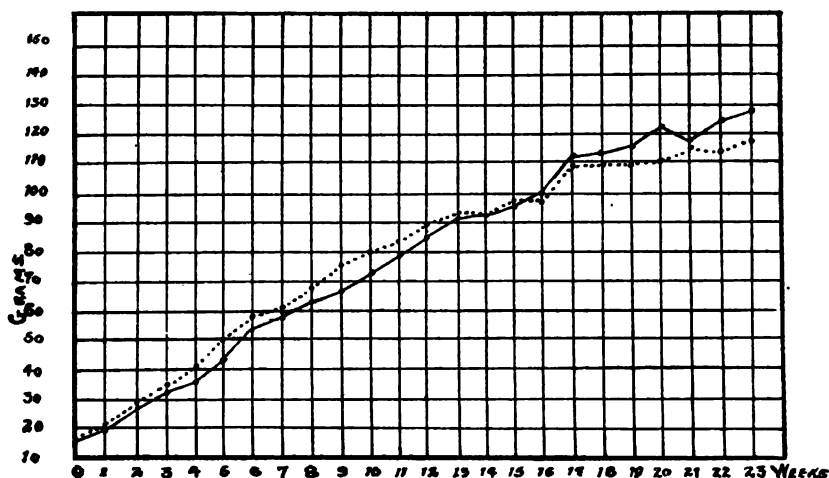


Fig. 2. Composite weight curve of female rats. Eight controls——, nine splenectomized..... Operated at 0 when fourteen days of age and carried through a period of twenty-three weeks.

TABLE 2

*Female rats*

Two rats: no. 27, an operated control; no. 30, a splenectomized animal. These animals were operated at fourteen days of age, have their weights included in the composite weight curves of figure 2, and were carried through a period of twenty-four weeks.

				DATE OF OPERATION					
				2/8/19	2/15	2/22	3/1	3/8	3/15
Rat 27. Operated control .....				11	18.5	22.5	26.5	29	33
Rat 30. Splenectomized .....				13	21	30	35	42	46
	3/22	3/29	4/5	4/12	4/19	4/26	5/3	5/10	5/17
Rat 27.....	39	45	51	58	60	67	72	74	72
Rat 30.....	60	67	76	94	87	92	98	97	99
	5/24	5/31	6/7	6/14	6/21	6/28	7/4	7/11	7/18
Rat 27.....	70	73	79	80	82	82	85	98	104
Rat 30.....	109	115	109	109	109	114	112	111	112

All weights are in grams.

mals have an average that exceeds the operated controls during the period of growth prior to maturity. It may be seen that the female rats gain in weight at a rate equivalent to the gains made by the male rats until they are about fifty days of age, at which time the male rats gain at a slightly faster rate. This is the normal manifestation shown by normal unoperated rats as observed by Donaldson, Dunn and Watson (8).

In two litters of rats weight records of one female and three male unoperated rats were made as a control upon operative procedure.

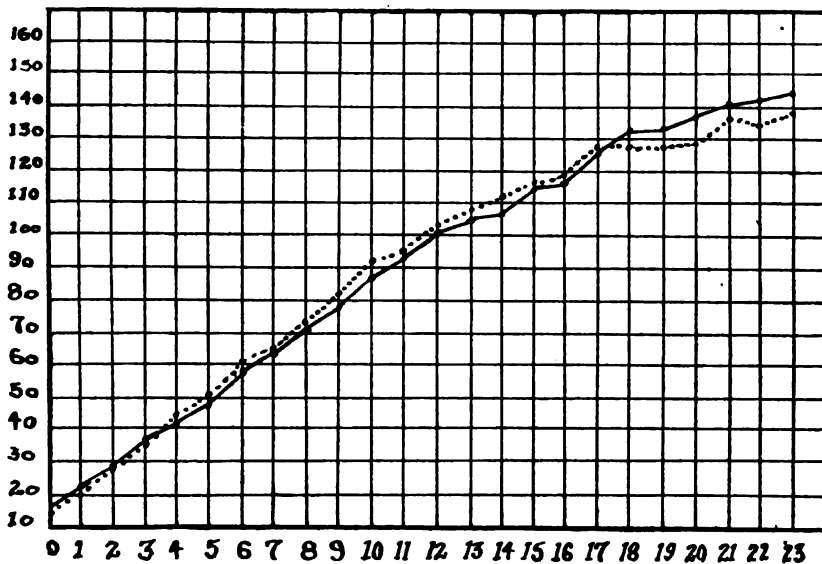


Fig. 3. Composite weight curve of all rats. Five male and eight female operated controls —; eight male and nine female splenectomized..... Operated at 0 when fourteen days of age and carried through a period of twenty-three weeks.

The weights were below those of the operated animals, significant of the negligible influence that operation had upon growth of the young animals.

In figure 3 there is a composite weight growth curve of all splenectomized and operated control rats (male and female). In this graph it is evident that the weight curves are almost identical during the period of growth prior to maturity.

*Rabbits.* Figure 4 contains the composite weight growth curves of four splenectomized and four operated control male rabbits. In table 3 the weights of a typical set of animals from this group and also the weights of an unoperated control are recorded. In figure 5 there are the composite weight growth curves of four splenectomized and three operated control female rabbits. In table 4 a typical set from these series is recorded.

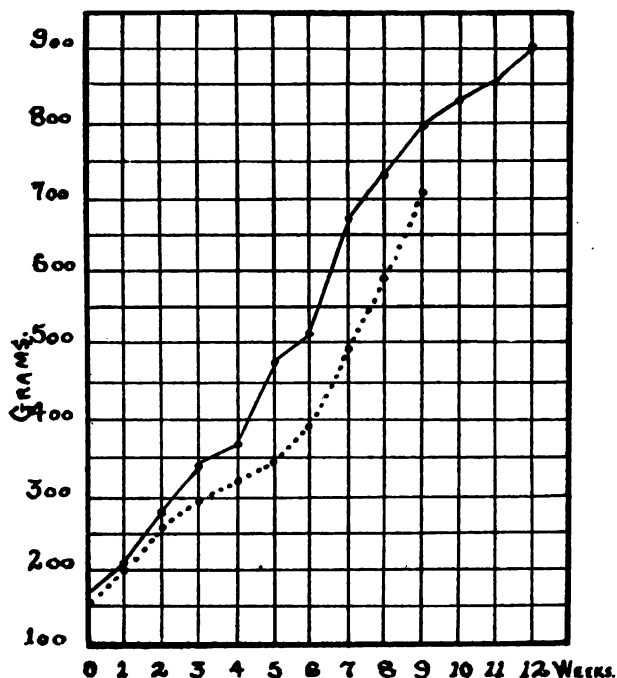


Fig. 4. Male rabbits. Composite curve of four operated controls — and four splenectomized rabbits..... Operated at 0 when fourteen days of age and carried through a period of twelve weeks.

It may be noticed that the general average of the male operated controls is above that of the splenectomized male rabbits and that the average of the female splenectomized rabbits is above that of the female operated controls. The differences are too small, considering the difference in their weights at the beginning of the records, to attempt an explanation through any effect the spleen may have upon metabolism.

TABLE 3

*Male rabbits*

Three rabbits: no. 5, a splenectomized animal; no. 6, an operated control, and no. 7, an unoperated control. The operated animals were fourteen days of age at time of operation and have their weights included in the composite weight curves of figure 4. The unoperated control was weighed at fourteen days of age, and thereafter weights were taken each week as were those of the operated animals.

	DATE OF OPERATION						
	1/24-19	1/28	2/6	2/12	2/19	2/22	3/5
Rabbit 5. Splenectomized.....	144	156	227	300	290	340	360
Rabbit 6. Operated control.....	174	195	284	310	357	352	390
Rabbit 7. Unoperated control...	145	202	292	365	359	342	375

All weights are in grams.

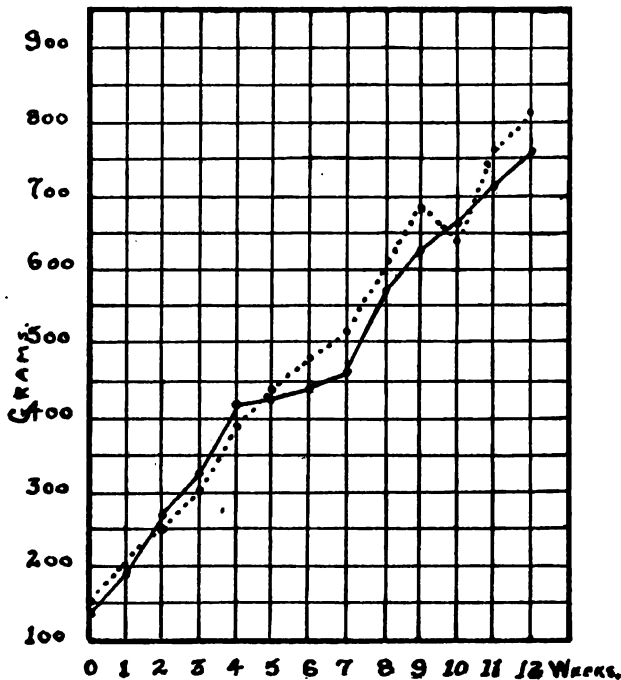


Fig. 5. Female rabbits. Composite curves of three operated controls — and four splenectomized..... Operated at 0 when fourteen days of age and carried through a period of twelve weeks.

In figure 6 are given the composite weight growth curves of all the male and female rabbits. Here it is evident that there is practically no difference in the growth curves of the splenectomized and operated control animals, especially when it is taken into consideration that the average of the operated controls was 20 grams above the weight of the splenectomized animals at the beginning of the records.

*Kittens.* In figure 7 there is the record of the weights, over a period of nine weeks, of kittens operated at fourteen days of age. Their record although irregular has the same general tendency as with the rats

TABLE 4

*Female rabbits*

Two rabbits: no. 11, a splenectomized animal; no. 9, an operated control. These animals were operated at fourteen days of age, have their weights included in the composite weight curves of figure 5, and were weighed through a period of thirteen weeks.

		DATE OF OPERATION					
		3/9/19	3/15	3/22	3/29	4/5	4/12
Rabbit 11. Splenectomized.....		155	182	239	319	382	512
Rabbit 9. Operated control.....		136	163	224	247	299	330
	4/19	4/26	5/3	5/10	5/17	5/24	5/31
Rabbit 11.....	506	602	614	634	507	672	725
Rabbit 9.....	307	346	425	420	443	465	470

All weights are in grams.

and the rabbits, that there is a similar gain manifested by splenectomized animals as with the operated control animals. In other kittens operated at seven weeks of age the same relative curves were observed in the splenectomized animals as in the operated control kittens.<sup>2</sup>

<sup>2</sup> Of the first litter of two kittens the splenectomized kitten did not gain in weight as did its control mate. It died about the tenth week weighing 260 grams. Its mate at that time weighed 800 grams. At the time of operation each weighed about 150 grams. Necropsy revealed congenital (?) absence of the thymus of the splenectomized animal. It is worthy of note that the splenectomized animal possessed adrenal glands which were twice as large and weighed almost twice as much as the adrenal of the control animal which was sacrificed for examination on the day of death of the other.—A. B. L.



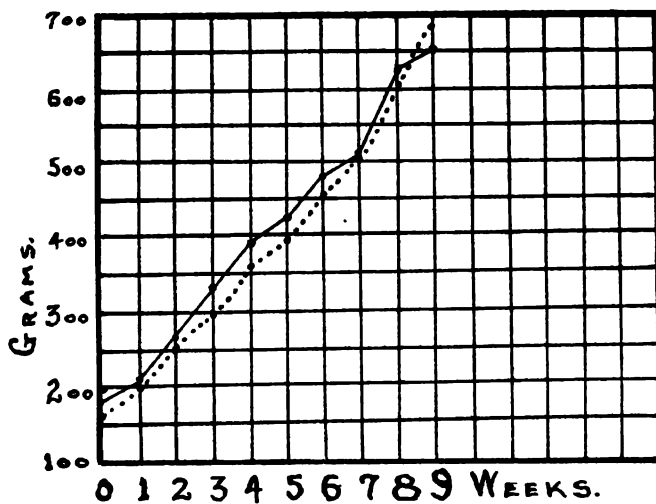


Fig. 6. Composite curves of all rabbits. Four male and three female controls —; four male and four female splenectomized.....

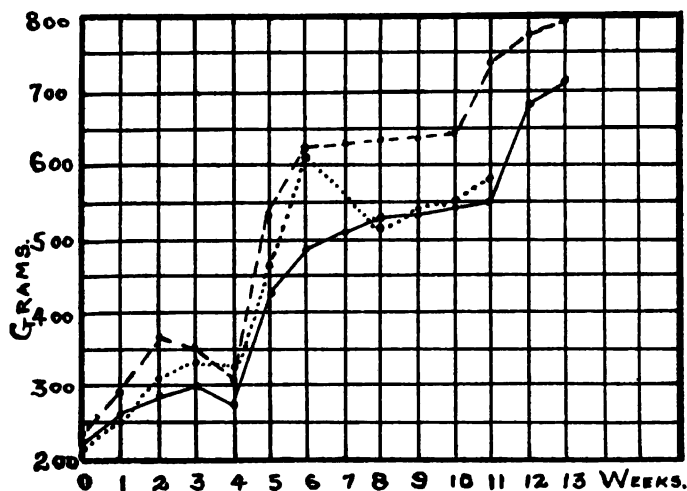


Fig. 7. Weight curve of kittens. One male control —; one male splenectomy —; one female splenectomy..... Operated at 0 when fourteen days of age and carried through a period of thirteen weeks.

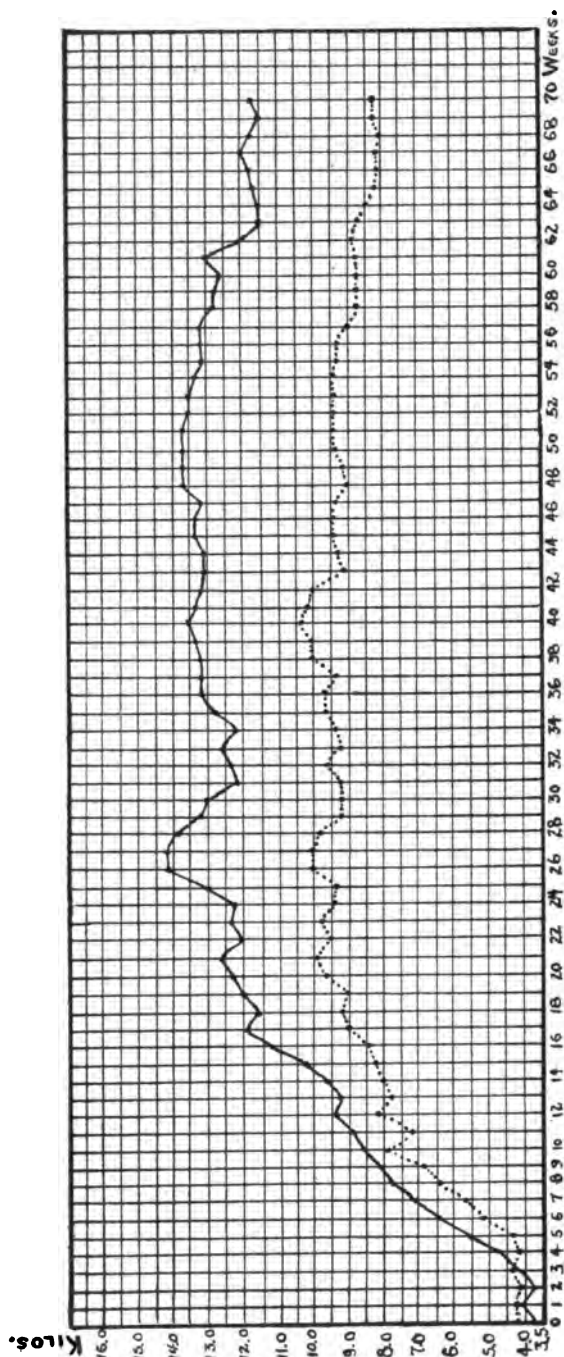


Fig. 8. Weight curve of dogs. Male operated control —; male splenectomized..... Operated at 0 when twenty-eight days of age and carried through a period of seventy weeks.

*Puppies.* Figure 8 of the dogs over a period of seventy weeks after operation at four weeks of age shows a marked difference in weight, but failure of others to withstand distemper after operation, and in light of former work on puppies (Tachigara and Takayi) there is insufficient data upon which any conclusions might be drawn in connection with growth. During the month following the operation these dogs had severe attacks of distemper. In addition to distemper the splenectomized animal had severe gastro-intestinal disturbances as was evidenced by vomiting and diarrhea. These factors may have had an inhibitory effect upon growth.

#### OTHER OBSERVED EFFECTS OF SPLENECTOMY IN THE YOUNG

##### *Blood changes*

*Dogs.* As this problem did not primarily deal with the blood picture, complete records have not been made. Blood counts were made on the dogs seven months after splenectomy. The splenectomized animal showed a general leucocytosis of 23,000 and moderate anemia (6,000,000 erythrocytes) compared to the counts made on the blood of control dog, which averaged 11,000 leucocytes and 8,600,000 erythrocytes. The same pipettes were used in making the counts on the two animals. The differential counts of blood cells from both animals gave percentages of cell types nearly equal.

*a. Resistance to hypotonic sodium chloride solutions.* *Dogs.* The blood corpuscles of splenectomized animals were found to be more resistant to hypotonic sodium chloride solutions than were the corpuscles of normal dogs.

Following the method of Karsner and Pearce (10) 0.1 cc. of corpuscular mass was placed in each of a series of tubes containing 3.0 cc. of sodium chloride solutions varying from 0.1 per cent to 0.5 per cent by differences of 0.025 per cent. Readings were made at the time the corpuscles were mixed with the salt solutions and eighteen hours later, having been kept in a refrigerator near ice. Corpuscles from two male dogs, ten weeks after being splenectomized at four months of age, gave 100 per cent hemolysis at 0.4 per cent and all solutions of lower percentage, with only a trace at 0.5 per cent. From a female dog, nine months after splenectomy at ten weeks of age, and from splenectomized male of figure 8, there was practically no hemolysis in solutions above 0.25 per cent, with no trace of hemolysis above 0.4 per cent. In four animals unoperated and varying in age from ten months to eigh-

teen months of age, there was complete hemolysis at 0.35 per cent and below, with no trace of hemolysis above 0.45 per cent. These results are in keeping with the observations made by Karsner and Pearce. They report similar results as having been observed by Bottazzi, Banti, Pugliese and Luzzati, and others.

TABLE 5

In this table there is a record of the coagulation time of the blood from three dogs. No. 1 was a male operated control, operated at four weeks of age and seventy-five weeks of age at this time. No. 2 was a male dog, splenectomized at four weeks of age, now seventy-five weeks of age. No. 3 was a female dog, splenectomized at ten weeks of age and now fifty weeks of age.

	SAMPLES	BLOOD DRAWN	FIRST EVIDENCE OF COAGULATION	FIRM CLOT	TIME OF COAGULATION
					<i>minutes</i>
Dog 1.....	1	9:38	9:42	9:45	7.0
	2	9:38	9:42	9:45.5	7.5
	3	9:38	9:42.25	9:46	8.0
	4	9:38	9:43	9:46	8.0
	5	9:38	9:43	9:46	8.0
	Average time .....				
Dog 2.....	1	10:09	*	10:10	1.0
	2	10:09	*	10:10	1.0
	3	10:09	*	10:10	1.0
	4	10:09	*	10:10	1.0
	5	10:09	*	10:10	1.0
	Average time less than 1'.....				
Dog 3.....	1	2:55	2:56	2:57.5	2.5
	2	2:55	2:56	2:57	2.0
	3	2:55	2:56	2:58	3.0
	4	2:55	2:56	2:58	3.0
	5	2:55	2:56	2:58	3.0
	Average time .....				

*Rabbits.* Corpuscles from a female rabbit, ten months after splenectomy at two weeks of age, were found to be no more resistant to hypotonic sodium chloride solutions than were the corpuscles of an operated control male rabbit of the same age.

*b. Coagulation time. Dogs.* In drawing blood from the ear and saphenous veins, the blood from splenectomized animals clotted much quicker than that from operated controls and normal animals. In table 5 the decided difference may be seen between the time of coagulation of blood in an animal splenectomized early, one splenectomized later, and the blood from a normal operated control animal.

Upon centrifuged blood of dogs of figure 8, it was found that corpuscles were decreased and serum increased in the splenectomized animal. The percentages were: corpuscles, 31.12 per cent; serum, 68.87 per cent; while in the control there was 58.66 per cent corpuscles and 41.33 per cent serum.

*Rats.* In seven male controls, seven male splenectomized, three female controls and four female splenectomized rats, there was no detectable difference in the time of the coagulation of their blood. Two samples of 1 cc. each were taken from the tail and the heart. Coagulation of the blood occurred in one minute.

*Rabbits.* In rabbits the coagulation time was determined of the same two as are recorded under "resistance to hypotonic solutions." The time of coagulation in five tests was found to be the same for each animal. These two animals lived longer than those that are recorded in the graphs.

#### *Appearance and temperament*

*Rats.* No difference in appearance and temperament could be observed between the splenectomized animals, the operated controls and the unoperated controls. Other people in being allowed to examine the rats and questioned as to what difference they might observe, could not differentiate between splenectomized and control animals.

*Kittens.* In the kittens a difference in temperament but not in appearance was observed. The splenectomized animals were more active and pugnacious, but as cats are very emotional animals no particular importance was attached to this difference.

*Dogs.* In appearance the splenectomized animal was emaciated, although fed an equal amount of food as the control. In temperament and activity both dogs responded alike. The splenectomized animal contracted mange at two different times while the operated control was resistant, while living under the same conditions.

*Fertility*

*Rats.* Two female splenectomized rats gave birth to litters of rats. The male in each instance had been completely splenectomized. The mothers cared for their offspring in a manner comparable to normal rat mothers.

*Rabbits.* A female splenectomized rabbit gave birth to a litter of rabbits. The male was an operated control. The young rabbits were well cared for.

*Dogs.* The male splenectomized dog of figure 8 was known to have copulated with a female animal which had been splenectomized at ten weeks of age. Two weeks later the mammary glands of the female became noticeably enlarged simultaneously with which there was a fall in the leucocytic count from 25,000 to 18,000. Two weeks later the swelling of the mammary glands disappeared and the leucocytic count rose to 29,000. No pups were ever delivered. Histological examination of the male's testes showed a very active spermatogenesis.

*Macroscopical and microscopical examination of tissues*

*Rats.* Macroscopic examination of the rats revealed no changes of especial note save that in most of the splenectomized animals there was red bone marrow in the femur. There were several of the control animals that showed traces of red in the femur, so that this finding was not limited to the femur of the splenectomized animals alone. In three of the splenectomized animals small bits of splenic tissue were found. It was interesting to note that these animals did not grow as rapidly as did the animals that had complete removal of the spleen or were only control animals.

Microscopically the only difference that was detected was in the bone marrow. The bone marrow of the splenectomized rat was very rich in cellular elements composed principally of myelocytes, erythrocytes, mononuclear cells and a few multinucleated cells. The fat content composed about one-third of the marrow. The bone marrow from the operated control was very cellular, composed of cells very similar to those of the splenectomized animal. Two-thirds of the marrow was composed of fat.

*Rabbits.* Macroscopic examination revealed a liver in the splenectomized female that weighed more than in the male control although the control weighed more than the splenectomized animal. The

difference in body weight was the normal difference between a male and a female. The marrow of the femur of the splenectomized animal was streaked with red, while it was distinctly yellow in the control.

Microscopically the liver of the splenectomized animal was marked with many Kupffer cells containing erythrocytes in different stages of disintegration. The bone marrow was composed of one-third fat. The remaining portion consists of many multinucleated lymphoid cells, myeloblasts, mononuclear lymphoid cells, nucleated erythrocytes, erythrocytes and a few endothelial and giant cells. The mononucleated lymphoid cells appear very much like splenic cells. The liver of the operated control was moderately cirrhotic (probably explaining difference in weight), and contained a few Kupffer cells in which there were erythrocytes. The bone marrow was composed of nearly all fat, with a few areas near the periphery containing a few lymphoid cells and myelocytes.

*Kittens.* Macroscopic examination revealed no observable changes. The bone marrow was distinctly yellow in the femur of the splenectomized animal as it was also in the control. No microscopic examination was made.

*Dogs.* Splenectomized animal: Macroscopically the most marked changes appeared in the lymph glands, bone marrow and in the weight of the liver which was 30.9 per cent of the body weight. The mesenteric and retroperitoneal lymph glands were distinctly red in color. The inguinal, axillary and popliteal glands were enlarged and white and firm in consistency. The intestine gave evidence of only *one* extremely atrophied Peyer's patch. The bone marrow was distinctly red in color and fairly firm in consistency.

Microscopically the liver contained many Kupffer cells in which there were many erythrocytes and a few leucocytes. The atrophied Peyer's patch was the seat of very little lymphoid tissue in the submucous layer. The mesenteric lymph glands in the periphery contained regularly arranged whorls of lymphoid tissue. In the medullary portion there were smaller and less regular whorls of the same tissue. There were marked sinuses in this portion containing few lymphoid cells, and many endothelial cells packed with erythrocytes, and a few leucocytes. There were also many multinucleated lymphoid cells present in these sinuses. The inguinal lymph glands were composed of more irregular lymphoid tissue than that in the mesenteric glands. There were several endothelial cells containing erythrocytes and there were many more multinuclear lymphoid cells present than were in the mesenteric

glands. The bone marrow was densely packed with cellular material interposed with small amounts of fat. The cellular element was composed of erythroblasts, erythrocytes, myeloblasts, mono- and multi-nucleated lymphoid cells and a few giant cells. The testes showed a very active spermatogenesis.

Operated control: The lymph glands were not enlarged, the bone marrow was distinctly yellow in color and the liver composed 23.2 per cent of the body weight.

Microscopically the lymph glands were rather compact and consisted of mononucleated lymphoid cells. The liver contained Kupffer cells but not to the marked degree that they were found in the liver of the splenectomized animal. The bone marrow was composed of a great amount of fat with very few mononuclear cells and myeloblasts interposed. The testes appeared very similar to those of the splenectomized animal both in structure and in showing a very active spermatogenesis.

#### DISCUSSION

In this work there are results from rats and rabbits in numbers large enough to permit of conclusion of the effect of splenectomy upon growth. It would not be justifiable in drawing conclusions upon the two series of kittens, although their weight curves are similar to the curves of the rats and the rabbits. In the dogs there were early influences of distemper and gastro-intestinal disturbances that present a factor that was not ruled out by other animals. However from the carefully prepared protocols of Tachigara and Takayi it is suggested that puppies would present weight curves similar to the curves of the rats and rabbits. Mann, in speaking of rabbits, dogs and kids, says: "Our results show that some animals from which the spleen has been removed quite early in life develop in the same fashion as the unoperated controls; furthermore, no definite specific change in the development due to the loss of the spleen was observed in any of the animals" (10).

The resistance shown by the corpuscles against hypotonic sodium chloride solutions is found to be true of animals operated while young as well as adult splenectomized animals. This phenomenon suggests that the spleen may have the function of preparing the erythrocytes for destruction.

The quickened coagulation time of the blood from splenectomized dogs may be a protective means against loss of blood by hemorrhage



during the time of anemia. The results would suggest that the earlier an animal is splenectomized, the more rapid is the time of coagulation. It may be due to the general leucocytosis or to an increased amount of fibrinogen which may account for the large amount of fibrin secured when the blood from splenectomized dogs is defibrinated. Increased fibrinogen may be due to a greater production by the liver in the absence of the spleen. No other mention of coagulation time of blood from splenectomized animals was found in a review of the literature save by T. G. Orr (11) working on rabbits. He reports that in twelve splenectomized rabbits there was no change in coagulation time as compared to normal rabbits. Coagulation time of the blood from rats and rabbits was found to yield no important differences in our own series.

In activity and temperament no difference was observed in rats, rabbits or dogs, but a slight difference in kittens was observed. This may be due to some individual variation rather than due to any effect following removal of the spleen.

Loss of fertility was suggested by the female splenectomized dog's failure in bearing any pups; but the active spermatogenesis of the male's testes and the swelling of the mammary glands at an early stage after copulation indicates that some other factor during early pregnancy may have had an inhibitory influence upon the development of any embryos. In rabbits it was clear that the female was not sterile. In rats it was evident that neither male nor female was sterile.

Histological examination indicates that the function of the spleen is in some way connected with the blood. This is evidenced by large hemolymph glands especially in the mesenteric region, which suggests that if it is due to a compensatory hyperplasia following splenectomy, it is important that such a function should be produced in the portal circulation. The bone marrow changes were rather striking. It may be that in disturbing a portion of the blood apparatus there is a compensatory functioning of other portions. The bone marrow through the different types of cells present, indicates that there is a process of erythrogenesis and leucogenesis taking place, while an increase of the Kupffer cells and endothelial cells of lymph glands indicates a process of blood destruction.

Changes in lymph glands and in the bone marrow similar to the above have been described by Pearce and Pepper (12) and by A. S. Warthin (13).

## CONCLUSIONS

1. The spleen is not essential to the life of young rats, rabbits, kittens and puppies.

2. Splenectomy has a negligible influence upon growth of rats and rabbits. It is suggested from the weight curves of the kittens that the same is true of these animals. Results of Tachigara and Takayi and Mann would indicate the same as being true of dogs.

3. Operative procedure has no debilitating effect upon the growth of young rats.

4. In dogs splenectomized while young there is a hastening of the coagulation time of blood. The younger the animals have their spleens removed, the more rapid is the coagulation time of the blood.

5. Corpuscles of young splenectomized dogs have the same property of more strongly resisting hypotonic sodium chloride solutions, than control and operated control animals, as do animals splenectomized after having reached maturity.

6. Accompanying the removal of the spleen there is an increase of Kupffer cells in the liver and changes in lymph glands and bone marrow that indicate a compensatory function on the part of these tissues in supplying a missing factor in blood formation and destruction.

7. Male dogs, male and female rats, and female rabbits are not rendered sterile through the removal of the spleen while these animals are young.

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# THE IMPORTANCE OF VAGAL AND SPLANCHNIC AFFERENT IMPULSES ON THE ONSET AND COURSE OF TETANIA PARATHYROPRIVA

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## INTRODUCTION

The object of this work was to determine, if possible, whether the afferent impulses which reach the central nervous system through the vagi and the splanchnic nerves have any influence on the onset and course of parathyroid tetany. That these might be of some importance was suggested to us by the great frequency with which tetany and gastro-intestinal disorders are associated clinically. Thus Osler (1) in quoting Frankl-Hochwart on the etiology of tetany in adults describes the "tetany of gastric and intestinal disorders, as dyspepsia, gastrectasis, diarrhoea, and helminthiasis," and again says that in children "tetany bears a definite relation to gastro-intestinal disorders." Paton, Findlay and Watson (2) mention this relationship in their classic on the parathyroids but do not seem to attach any significance to it.

McCann (3) attributes the tetany to the alkalosis resulting from the stagnation of the acid secretion in the stomach but Osler (op. cit.) says that "the type associated with dilatation of the stomach is rare, only thirty instances being reported" and hence this would not explain the great majority of cases. Ivy (4), from work done in this laboratory, reports that in eight dogs with duodenal fistulas which allowed everything the dogs drank together with the gastric juice to drain to the outside, only one developed tetany. This animal was advanced in pregnancy and the tetany he attributed to eclampsia of pregnancy. However Rodman (5), Bene (6) and others consider gastric ulcer and dilatation to be more closely associated with tetany.

Oppenheim (7) in discussing tetany says:

Adults who suffer from gastro-intestinal affections are sometimes affected. . . . It has not been definitely decided whether the toxic agent is the cause of the spasm or whether the spasm has a reflex origin. The fact that it has occa-

sionally occurred directly after the use of a stomach pump or the passing of a tube or after profuse vomiting seems to point to a reflex origin, and the observation—which cannot be implicitly relied upon—that the spasm has disappeared after the evacuation of entozoa confirms this theory. It is remarkable that Gerhardt was able in a case of tetany with dilatation of the stomach to bring on the spasm by slight percussion of the stomach.

In this laboratory it has been frequently observed that an attack of tetany may be precipitated in parathyroidectomized animals by the passage of a stomach tube, other sensory stimuli also having the same effect.

Carlson and Jacobson (8) and later Friedman (9) also recorded the great frequency with which hyperemia, hemorrhages and ulcers of the stomach and intestinal mucosa are found in the post-mortem examination of parathyroidectomized animals and were reminded of the relationship between gastro-intestinal disorders and tetany in man, stating further that "it is not known to what extent this tetany is due to toxic products absorbed into the blood or to irritation of afferent nerve endings, but in late years numerous attempts have been made to bring all types of clinical tetany within the category of parathyroid tetany." A priori we would reason that if irritation of the afferent nerve endings in the gastro-intestinal tract is ever of importance in tetany, it should be in that of parathyroid origin for in this condition we have not only these alimentary disorders but also a hyper-irritable condition of both the central nervous system and the peripheral nerves, as has been shown most completely by Paton, Findlay and Watson (*op. cit.*). Before undertaking this work it was thought that examination of the literature upon the effect of section of the cord at different levels might throw some light upon the subject but the work of Biedl (11), Falta and Rudinger (11), Paton, Findlay and Watson (2), Mustard (12), Carlson and Jacobson (8), and of Luckhardt, Sherman and Serbin (13), is at such variance that no conclusions on this point can be drawn.

#### EXPERIMENTAL METHODS

Two methods of procedure have been followed in these experiments. The first consisted in the determination of the effects of double vagotomy and splanchnectomy on the onset and course of parathyroid tetany and the second in the determination of the effect of gastro-intestinal irritation by means of a drastic, i.e., croton oil, upon the onset and course of parathyroid tetany. It was recognized of course that this artificial irritation is not strictly analogous to the normal irritative condition. Dogs were used exclusively in the work.

The technique of the first method was divided into three stages. In the first operation the thorax was entered on the left side between the sixth and the seventh ribs. Here the thoracic sympathetic trunk was picked up and all the rami communicantes torn loose in the lower part of the thorax, care being taken to destroy all threads of nerve tissue running from the rami communicantes to the left splanchnic. This nerve trunk was then grasped with the fingers and jerked out from the diaphragm. After thus getting rid of the left splanchnic, the esophagus was picked up and the vagi, which are found lying on each side at this level, were sectioned. Care was taken here to make sure that all of the vagus fibers were cut. The closing of the thorax completed the operation. Ether anesthesia alone was used, no morphine being employed because of its depressing effect upon the respiratory center. Artificial respiration was unnecessary unless the thorax was allowed to remain open for too long a time, in which case asphyxiation resulted.

After a week or so when this wound was found to be well healed and the animal in good condition, the second operation was performed under morphine-ether anesthesia. This consisted in entering the abdominal cavity through a right lumbar incision and picking up the right splanchnic just after it pierces the diaphragm. After dissecting this down to the point where it branches out to enter the coeliac plexus, the nerve and ganglion were removed and the abdomen closed. This operation is not so depressing as has been supposed, the dogs appearing lively and in good condition within a few hours after the operation and usually live in the best of health, apparently suffering no ill effects at all. The success of the two operations was determined at the subsequent post-mortem examination.

After a week or ten days had again been allowed to elapse, a complete thyro-parathyroidectomy was performed, the glands being removed grossly with as much of the surrounding loose tissue as possible in order to avoid leaving behind any accessory parathyroid tissue in that region. The animals were kept under the same conditions as before the operation and on the same diet (bread and meat). The electrical excitability of the femoral nerve was determined before the final operation and the change afterwards followed although it was expected from the work of Paton, Findlay and Watson that double vagotomy and splanchnectomy would have no effect upon the change in the electrical excitability of the peripheral nerves even though it did have an effect upon the tetany.

The technique of the second method consisted also in complete thyro-parathyroidectomy in the manner just described, followed by the

daily administration of croton oil in varying amounts. The U. S. P. dose of this drug is 2 minims and hence the 2 to 4 minims given daily to the dogs produced very severe irritation. The animals were kept under the same conditions as those of the first series and in most cases the electrical excitability was followed here also.

#### RESULTS

The animals developed distemper so frequently that this proved to be quite a troublesome factor, especially in the first series. In a large proportion of the cases autopsy showed a terminal infection with pneumonia. It is quite conceivable that this depressing condition might alter the course of the tetany somewhat but its effect was probably similar in the two series.

*Effect of double vagotomy and splanchnectomy.* Table 1 gives a summary of the results obtained in the first series of experiments. Nine of the sixteen animals developed tetany, five showed depression only and two are still living. The failure of these last two animals, dogs 48 and 52, to show any symptom is probably to be attributed to the presence of accessory parathyroid tissue. Excluding these from consideration, nine out of fourteen animals, or 64 per cent, developed tetany and five showed depression only. In the series of thyro-parathyroidectomies on dogs reported by Paton and Findlay, twenty-six out of forty-two, or 61 per cent, showed tetany. The severity of the tetanic attacks seemed to be as great in these dogs as in normal parathyroidectomized dogs and even greater than in the dogs of series 2. The protocol of the dog showing the most pronounced tetany in this series is given in table 3. It will be noticed that this dog vomited frequently, which point is also of interest since it points to direct central stimulation as the cause of the vomiting.

*Effect of artificial gastro-intestinal irritation.* Table 2 gives a summary of the results obtained in the second series of experiments. Here eleven out of fourteen dogs, or 78 per cent, showed tetany and three depression only. Although this is a somewhat higher percentage than that of series 1, it is scarcely of significance, especially in view of the fact that the severity of the tetany in these animals did not seem to us to be quite so great nor the attacks so prolonged as in those of the first series. The protocol of the animal which showed the greatest tetany in this group is given in table 4. Although this animal showed pneumonia at autopsy while that of the first protocol did not, it lived longer in spite of the pneumonia and hence the comparison between the two is a rather fair one and illustrates the similarity between the two series.

TABLE I  
Effect of double vagotomy and splanchnectomy on parathyroid tetany

DOG	FIRST OPER.	SECOND OPER.	THIRD OPER.	ONSET	NO. ATT.	SEVER.	DEP.	E. E.	DUR. EXP.	REMARKS	AUTOPSY
				hours					days		
7	4/8	4/19	4/23	125	2	++	+	+	6		Pneumonia; br. rt. spl. intact
30	4/30	5/31	6/11	0	0	0	+	+	1½		Pneumonia; both oper. complete
32	5/1	5/15	6/11	67	2	++	+++	++	11½		Pneumonia; br. lft. spl. intact
33	5/1	5/14	6/11	35	3	+++	+++	++	8½	Groans continuously; encephalophoria	Slight pneumonia; br. left spl. intact; hem. in intestines
36	5/5	5/9	6/10	26	2	++	+	+	2½		Hyper. gastric mucosa, both oper. complete
38	5/10	5/13	6/10	41½	2	++	+	++	2		Both oper. complete; hem. in intestines
39	5/10	5/24	6/8	15	2	++	+	+	1½	Advanced pregnancy	Rt. spl. ima intact
40	5/13	6/4	6/10	0	0	0	+++	++	9	Slight tremors only	Pneumonia; thread of rt. spl. intact; hem. in intestines
44	5/17	5/23	6/14	0	0	0	+++	++	5½	Vomits, dis-temper	Pneumonia; both oper. complete
46	5/19	5/23	6/10	60	4	+++	+	++	11		Pneumonia; both oper. complete; hem. in duodenum
47	5/20	5/27	6/10	0	0	0	+++	+	3	Very sick, dis-temper	Pneumonia; both oper. complete; hyper. gastric mucosa
48	5/24	5/31	6/16	0	0	0	0	0		Living	Pneumonia; rt. spl. ima intact
49	5/24	5/27	6/14	0	0	0	+	+	7½		Both oper. complete; hem. in intestine
50	5/26	6/4	6/12	74	3	+++	+	++	5½		Both oper. complete; hem. in gastric mucosa
51	5/26	6/2	6/10	84	3	+++	++	++	6½	Vomits often	Both oper. complete; hem. in gastric mucosa
52	5/26	6/2	6/10	0	0	0	0	0		Living	
Average.....				58½	2½				5½		

Note. att.—attacks; sever.—severity; dep.—depression; E.E.—electrical excitability; dur.—duration; hem.—hemorrhage; hyper.—hyperemia; br.—branch; spl.—splanchnic; one + slight; two ++ mild; three +++ severe.

TABLE 2  
Effect of artificial gastro-intestinal irritation on the severity of parathyroid tetany

DOC	TRY- PAR. G. OIL	TOTAL DROPS	ONSET	NO.	SEVER.	DEP.	E. E.	DUR. EXP.	REMARKS	AUTOPSY
			hours	ATT.				days		
2	5/16	12*	29	2	+++	++		5½	Bld. stool; Vomits	Hem. in gastric mucosa
3	5/20	8	48	1	+	+		2½	Bld. stool	Slight pneumonia; hem. in intestine
4	5/20	20	94	1	+	+		4	Vomits	Pneumonia; hem. in intestine
5	5/22	8	48	1	+++	+		2½	Death in tet- any (?)	Hem. in gastric mucosa
6	5/22	8	24	2	+	+		2½	Vomits	Pneumonia; hem. in intestine
7	6/17	4	50	1	+	+	+	3	Death in tet- any (?)	Pneumonia; hem. in gastric mucosa
8	6/17	4	0	0	0	+	++	2	Vomits blood	Hem. in intestine
9	6/18	10	47	2	+	++	+	3½		Pneumonia; hem. in gastric mucosa
10	6/18	6	22	2	++	++	++	6		Pneumonia; hem. in gastric mucosa
11	6/21	18	0	0	0	+++	++	9½		Pneumonia; hem. in gastric mucosa
12	6/24	4	27	1	++	+	+	2½		Pneumonia; hem. in gastric mucosa
13	6/24	16	48	1	++	+++	++	8		Pneumonia; hem. in gastric mucosa
14	6/25	19	0	0	0	++	++	8½		Pneumonia; slight hem. in intestine
15	6/25	9	54	5	+++	+++	++	8		Pneumonia; hem. in intestine
Average.....			44½	1½				5		

\* Twelve grains aloes.

Note: c. oil—croton oil; att.—attacks; sever.—severity; dep.—depression; E. E.—electrical excitability; dur. exp.—duration of experiment; hem.—hemorrhage; one + slight; two ++ mild; three +++ severe.



TABLE 3  
*Protocol of animal 51, series 1*

DATE	HOUR	PROCEDURE	ELECTRICAL REACTION IN MILLIAMPERES			REMARKS
			KCC	ACC	AOC	
5/26	9:00 p.m.	1st op.				
6/4	10:00 a.m.	2nd op.				
6	3:00 p.m.		1.0	2.4		
7	11:15 a.m.		1.8	4.8		
9	8:30 p.m.		1.8	2.8		
16	11:00 a.m.	3rd op.				
	5:00 p.m.		0.8	1.6		
17	10:00 a.m.		0.8	1.2		
	5:00 p.m.		0.6	1.0		Vomits frequently
18	9:00 a.m.		0.4	0.6		Vomits frequently
	5:00 p.m.		0.4	0.6	0.6	
19	10:00 a.m.		0.4	0.5		Vomits frequently
	5:00 p.m.					Slight tremors
	8:00 p.m.					Tremor all evening but not severe
	11:15 p.m.					Mild spastic tetany, particularly in forelimbs
20	3:30 a.m.					Mild spastic and clonic tetany all limbs
	10:00 a.m.					Tetany continues, occasionally severe
	2:00 p.m.					Mild tetany continues
	3:30 p.m.					Tetany gone, tremors present
	5:00 p.m.					Mild tetany again
	9:00 p.m.					Mild tetany, occasionally severe
21	9:00 a.m.					Tremors
	10:00 a.m.					Mild tetany, at times severe
	1:00 p.m.					Mild tetany continues
	5:00 p.m.					Mild tetany has continued all afternoon frequently being severe
	9:00 p.m.					Depressed
22	10:00 a.m.					Depressed
	8:00 p.m.					Found dead

## Autopsy:

1. No pneumonia
2. Primary and secondary operations had been complete
3. Slight petechial hemorrhages in gastric mucosa
4. Other organs normal

TABLE 4  
*Protocol of animal 15, series 2*

DATE	HOUR	PROCEDURE	ELECTRICAL REACTION IN MILLIAMPERES			REMARKS
			KCC	ACC	AOC	
6/21	5:00 p.m.		0.5	1.2		
23	10:00 a.m.		0.8	1.0		
25	9:00 a.m.		0.4	1.2	0.8	
	11:00 a.m.	Thy-par				
	5:00 p.m.	2d C. oil				
	8:00 p.m.		0.4	0.8	0.8	
26	9:00 a.m.	2d C. oil				Slight tremors
	12:00 m.					Mild tremors
27	9:00 a.m.	2d C. oil				
	5:00 p.m.					Severe tetany
	10:00 p.m.					Quiet
28	9:00 a.m.	3d C. oil				Severe tetany
	9:00 p.m.					Tetany
29	9:00 a.m.					Severe tremors
	8:00 p.m.					Severe tetany
30	1:00 a.m.					Mild tetany
	10:00 p.m.					Alternating severe tremors and mild tetany
	4:00 p.m.					Severe tremors. Depressed
	9:00 p.m.					Slight tetany
7/1	9:00 a.m.					Depressed
	12:30 p.m.					Mild tetany
	10:30 p.m.					Mild tetany with intervals of depression
2	8:30 a.m.					Depressed. Occasional tre- mors
3	9:00 a.m.					Very depressed
	1:00 p.m.					Found dead

## Autopsy:

1. Extensive pneumonia involving both lungs
2. Hyperemic gastric mucosa with petechial hemorrhages throughout small intestine.
3. Other organs normal

## DISCUSSION

In the first table it will be seen that there is no relationship between the dogs which showed tetany and those in which the primary and secondary operations were incomplete. Thus in dogs 40 and 49 the opera-

tions were incomplete and the dogs showed no tetany, while in dogs 36, 38, 50 and 51 the operations were complete and yet the dogs showed tetany. Similarly in the second series there seems to be no relationship between the amount of irritation produced and the onset, severity or number of attacks of tetany. Comparison of the two series shows further that although the average length of time before the onset of tetany was somewhat shorter in the second series and the average length of life a little less, the difference is not enough to be significant especially in view of the fact that the severity of the tetany was somewhat greater in the first series.

The vomiting recorded in the protocol in table 3 and observed also in one other dog in the first series is rather interesting. Carlson and Jacobson observed that in parathyroid tetany "the vomiting is frequently severe and persistent, even in subjects taking no food." Since it occurs in animals with both the vagi and the splanchnics sectioned, one must conclude that possibly this vomiting is of central origin. Subcutaneous morphine-atropine injections preparatory to anesthetization for the tertiary operation was observed to have a similar effect in these animals.

Carlson and Jacobson also called attention to the groaning with evidences of abdominal tenderness so frequently seen in parathyroid tetany dogs. This was shown in our first series by no. 33 especially which was observed to groan continually and although autopsy showed a very small twig of the left splanchnic to be intact, it hardly seems probable that this groaning is due to stimulation of splanchnic or vagus afferent endings.

The results obtained in this work, then, have been entirely negative. The vagi and splanchnics are of no importance in parathyroid tetany in dogs. To reason from this that these nerves are of no importance in tetanias associated with gastro-intestinal disorders is, of course, unsound. However, the present finding does not support the theory of a nervous relationship between the two conditions, but rather lends favor to the view of a chemical relationship.

#### CONCLUSIONS

1. Double vagotomy and splanchnectomy have no influence upon the onset and course of tetania parathyropriva.
2. Artificial gastro-intestinal irritation has no influence upon the onset and course of tetania parathyropriva.

The above research was done at the suggestion of Dr. A. B. Luckhardt and it is a pleasure here to record my gratitude for his constant interest and helpfulness. The technique was developed by Mr. Raymond Householder in a series of preliminary experiments, for which I am greatly indebted.

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